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MYXOSPORIDIA FROM FISHES OF THE GENUS *ESOX*

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As parasites of the common pike, *Esox lucius* Linn., Kudo (1920: 29) lists five species of the genus *Henneguya* (*H. psorospermica, H. oviperda, H. lobosa, H. peri-intestinalis, H. schizura*), one species of the genus *Myxobolus* (*M. anurus*), and one species of the genus *Myxidium* (*M. lieberkühni*). Studies on the same host fish in the United States and Canada have shown only the form *Myxidium lieberkühni* Bütschli present. This has been especially noted by Mavor (1916a, 1916b). Gurley (1893) reported that Müller failed to find any infections of *Henneguya schizura* (Gurley) Labbé in the pike he examined in the United States, while Kudo (1934: 11) notes two pike from Illinois waters negative for any myxosporidian infection. In the summer of 1937 the writer examined 27 *E. lucius* from various waters of the Upper Peninsula of Michigan with negative results.

The only other reports of myxosporidian infections of *Esox* spp. were noted by Kudo (1918) when he showed three *Esox* spp. from the Woods Hole area to be negative and again (Kudo, 1921) when he reported *Myxidium lieberkühni* in 13 of 13 *Esox reticulatus* (probably *E. niger* Le Sueur) of New York State and also described *Wardia lucii* from 2 of the 13 fish.

MATERIALS AND METHODS

Material from *Esox niger* Le Sueur was collected by the writer in March, 1936, from the Middle River, Maryland, about 15 miles south of Baltimore, Md. The writer is indebted to Dr. Justus F. Mueller, Syracuse University, for material from *Esox masquinongy* (Mitchill) taken in Chautauqua Lake, N. Y., June, 1937. All material was studied after preservation in 4 per cent formalin. Descriptions are made from aqueous solutions of the formalin material in hanging drop preparations of the type described by Nemeczek (1926). Studies were made with oil immersion (1.9 mm) objective and 15× ocular in the determination of the spore characters. Dobell's and Lugol's iodine solutions were used for generic determinations, and further observations were made on slides stained with Giemsa's stain and with Ehrlich's acid hematoxylin.

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Henneguya nigris n. sp.

(Fig. 1)

Habitat: The species is found in cysts lying along the central portion of the gill filament of *Esox niger* taken from Middle River, Maryland, 15 miles south of Baltimore, Md., March, 1936. Also found in *Esox masquinongy* of Chautauqua Lake, N. Y., taken June 14, 1937. The cysts were much larger and more obvious in *E. niger* while on *E. masquinongy* they were smaller and located in the outer third of the gill filament. One of three *E. niger* was infected and the species was found in both collections of *E. masquinongy.*

Vegetative form: Mature and partly mature cysts were found. The larger cysts on the gills of *E. niger* were irregular in shape and lodged at about the middle of the gill filament. The cysts were elongated, 4 to 5 mm by 1 to 2 mm in size, the longest dimension of the cyst lying with the axis of the filament. The cysts of *E. masquinongy* were smaller, averaging 2 by 1.5 mm. Cyst wall only slightly elastic, opaquely white and apparently strongly bound by connective tissue reaction of the host. Pansporoblasts disporous. Polysporous.

Spore: Spore broadest at the capsular end of spore, tapers rapidly to the end of the sporoplasm and from that point both valves are elongated into fine tapering processes (Fig. 1). Spindle-shaped laterally. Tails are noticeably bent or curved to one side; the shell thin and smooth; valves symmetrical. Sutural line is faint and sutural ridge slightly raised. Capsular end of the spore is definitely flattened, with a slight depression at the capsular openings.

Two sub-equal polar capsules are present, comparatively short and not convergent. The filaments are wound in the capsules in 5 to 6 turns. Sporoplasm is large, occupying about 4/5 of the spore. The plasm is finely granular and contains numerous large granules or inclusions; a medium sized (4 by 3 microns) iodinophilous vacuole; and in stained preparations two strongly staining nuclei may be seen.

Dimensions of unstained spores are as follows: total length 45 to 60 microns (57 microns); length of spore, excluding processes, averages 22 microns; breadth of spore 7 to 8 microns; thickness 4 to 5 microns; polar capsules 5 to 6 by 2.5 microns. The ratio of length of spore to length of tails is 1 to 1.5.

Remarks: This species varies in spore form from all species of *Henneguya* previously described. The elongated, pear-shaped spore body, with the broadest portion at the capsular end, is specifically distinctive. The divergent polar capsules are also peculiar to this species of *Henneguya.* Other forms, where such data is present, have the polar capsules more or less convergent.

Henneguya acuta n. sp.

(Figs. 2–3)

Habitat: The cysts were found between the branchial lamellae of both collections of *Esox masquinongy* taken from Chautauqua Lake, N. Y., June 14, 1937. The cysts were localized in the basal 1/3rd of the gill filament.

Vegetative form: The whitish, opaque cysts (2 to 3 by 1 to 2 mm) were found in both mature and semi-mature stages. Smaller cysts contained an abundance of developing disporous pansporoblasts with the more advanced and mature stages near the center of the cysts. The cysts lie in the connective tissue between the lamellae, the axis of the cysts at right angles to the axis of the gill filament.

Spore: Mature spores are broadly spindle-shaped in face view with the capsular end sharply pointed. Spindle-shaped in side view (Figs. 2 and 3). The shell is smooth and thin with the valves symmetrical, each of which is drawn out into a long tapering process. The sutural ridge is well developed and noticeably curved but the sutural line is not distinct.

Polar capsules in this species vary from the preceding by being normally convergent. Capsules are sub-equal, occupying about 1/5th of the spore cavity. The filaments may be made out much easier than in the above form; 8 to 9 coils

being visible. Sporoplasm is finely granular with distinct iodinophilous vacuole. Two nuclei are visible in normal, stained spores.

Dimensions of unstained spores: total length 33 to 66 microns (57 microns); length of spore excluding process 14 to 18 microns (16.5 microns); breadth 4 to 5 microns; thickness 4 microns; polar capsules 5 to 7 by 2 microns; iodinophilous vacuole 3 to 4 by 1.5 microns. Ratio of length of spore to length of tail is 1 to 2.4.

Remarks: Henneguya acuta more closely resembles *H. exilis* Kudo than other *Henneguya* sp. of similar form. *H. acuta,* however, is more sharply pointed at the capsular end and the proportions of spore body length to length of tail (1 to 2.4) are definitely different from those proportions for *H. exilis* (1 to 3.5).

Myxosoma cuneata n. sp.

(Figs. 6–7)

Habitat: Cysts are found under the dermis of the inner surface of the gill arch, from 3 to 7 cysts being found on each gill arch. Present in one of two collections of *Esox masquinongy* taken from Chautauqua Lake, N. Y., June 14, 1937.

Vegetative form: Whitish, spherical cysts of 2 to 3 mm in diameter were lodged about 1 to 2 mm below the surface. There was no elaboration of host tissues noticeable and upon dissection the cysts were found to be loosely set in the cavities formed by their growth. Cysts quite dense and brittle (in formol). All cysts found were mature to a degree that only a very few disporous pansporoblasts were to be found. Polysporous.

Spore: Ovate pyriform in face view, lenticular in side view (Figs. 6 and 7). Spore coat of medium density with a wedge-shaped marking arising from the base of the spore and extending to about the center of the sporoplasm. This marking is characteristically asymmetrical, the point terminating to the left of the mid line of the spore as viewed in face view. Sutural ridge heavy with a definite sutural line present.

Two sub-equal polar capsules are present. Normally convergent, they occupy between 1/3 and 1/2 of the spore. Filaments in 9 to 10 turns. Sporoplasm finely granular; iodinophilous vacuole absent. Two nuclei are visible in normal spores after staining.

Dimensions of unstained spores: total length 9 to 10 microns (10 microns); breadth 5 to 7 microns (6 microns); thickness 4.5 microns; polar capsules 4 to 6 by 1.5 to 3 microns. Capsules about twice in spore length.

Remarks: Myxosoma cuneata approaches a number of previously described myxosporidians in size and form but differs from all in carrying the wedge-shaped marking described above. *Myxosoma acuta* (Fujita) and *M. ovalis* (Davis) are both similar to the present form. However, these forms tend to be more circular in face view and lack any such marking as described.

Myxosoma muelleri n. sp.

(Figs. 4–5)

Habitat: Found in the connective tissue between the branchial lamellae of *Esox masquinongy.* Taken in two collections of this fish from Chautauqua Lake, N. Y., June 14, 1937. The distal third of the gill filament was apparently the favored portion of the gill.

Vegetative form: Present as white, oval cysts of 2 to 3 by 1 mm, superficially resembling the cysts of *Henneguya acuta.* Slight elaboration of host tissue about the cyst was noticed. Cyst coat moderate and quite resistant to pressure. All cysts contained mature spores and disporous pansporoblasts. Polysporous.

Spore: Bluntly ovate in face view, lenticular in side view with one valve somewhat larger (Figs. 4 and 5). Spore coat medium thick; two lateral and one basal marking present so that two quadrilateral plaques are apparent at the base of the spore. Sutural ridge moderate, sutural line distinct.

Polar capsules equal, slightly convergent but not crossing when studied in face view. Filaments in 8 to 10 turns. Capsules comparatively large, occupying about half of the spore. Sporoplasm finely granular; iodinophilous vacuole absent; one or two nuclei present in stained spores. In side view the sporoplasm may be seen to extend along one side of the capsules for about one half of their length.

Dimensions of unstained spores: total length 12 to 13.5 microns (13 microns); breadth 7 microns; thickness 4.5 microns; polar capsules 7 by 2.5 to 3 microns. Capsules are about twice in spore length.

Named for Dr. Justus F. Mueller, Syracuse University, to whom the writer is indebted for the material from *Esox masquinongy*.

Remarks: Myxosoma dujardini Thel., *M. lobatum* Nemeczek, and *M. funduli* Kudo are all somewhat similar in dimensions and shape to the present form. *M. muelleri* varies from these in that the base of the spore is narrower and there are present the definite markings on the spore coat as described.

SUMMARY

1. It is noted that although *Esox lucius* Linn. carries six species of histozoic myxosporidian parasites in European waters no histozoic forms have yet been reported infecting this host in American waters.

2. *Henneguya nigris* n. sp. is described from *Esox niger* Le Sueur and *E. masquinongy* (Mitchill). *Henneguya acuta* n. sp., *Myxosoma cuneata* n. sp., and *Myxosoma muelleri* n. sp. are described from *Esox masquinongy*.

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EXPLANATION OF PLATE

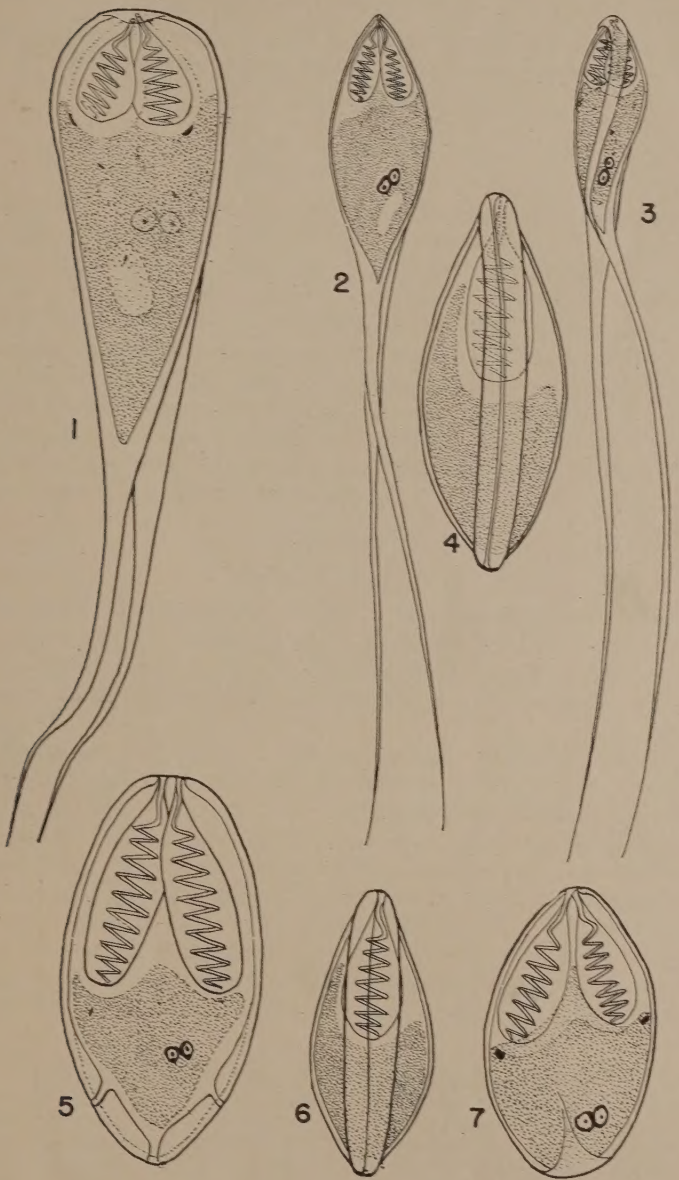
All drawings about 3,000× by aid of camera lucida.

FIG. 1. *Henneguya nigris*, front view.

FIGS. 2, 3. *Henneguya acuta*, front and side views.

FIGS. 4, 5. *Myxosoma muelleri*, side and front views.

FIGS. 6, 7. *Myxosoma cuneata*, side and front views.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THE MORPHOLOGY AND LIFE HISTORY OF *PSILOSTOMUM ONDATRAE* PRICE, 1931 (TREMATODA: PSILOSTOMIDAE)*

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McMullen (1938) recently described from the region of Douglas Lake, Michigan, a new cercaria which he named *Cercaria thomasi*. In its behavior and many of its morphological features it closely agreed with certain of the echinostome cercariae, but because of the absence of a cephalic collar and spines, and because it resembled the cercaria of *Psilotrema spiculigerum* as described by Mathias (1925), it was tentatively and correctly placed in the family PSILOSTOMIDAE. In the summer of 1938 while the writer was engaged in a study of the echinostomes of the Douglas Lake region, *C. thomasi* frequently was encountered and because of its apparent relation with the ECHINOSTOMIDAE, the determination of its life history was undertaken.

THE CERCARIA

(Figs. 2, 5–9)

McMullen's description of *C. thomasi* is in general agreement with my own observations. In order to have a more complete description with special attention to such characters as may be of value in a systematic treatment of the group to which it belongs, my own observations are given.

Throughout the summer of 1938 the cercariae were fairly abundant in collections of *Helisoma antrosum percarinatum* (Walker) from several areas in the region of Douglas Lake. Approximately 100 snails bearing this cercaria were brought into the laboratory. The cercariae always emerge during the early part of the night. They are very active swimmers and under average laboratory conditions they swim almost continuously for 12 hours or more. During this period there is no concentration of the cercariae but as they become weaker they become concentrated at the bottom, and if examinations were made only at mornings, this concentration at the bottom would appear to be a typical habit. Swimming

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activities are similar to those of the echinostomes. The body may either precede or follow the tail and usually is flexed ventrally while swimming. Frequently an individual will settle down on a smooth surface and undergo active creeping. During these creeping activities the body is constricted slightly just back of the acetabulum but not more so than in the case of any cercaria with a very protrusible acetabulum (Fig. 8).

Description. Relaxed, the body is elongate with the sides more or less parallel in the posterior two-thirds, tapered anteriorly; widest portion just anterior to acetabulum. Oral sucker powerful, terminal, oval in outline. Acetabulum in anterior part of posterior half of body, muscular, very protrusible, and provided with a single row of 79 to 81 minute hooks on its outer border (Fig. 5). Prepharynx fairly long, thin-walled. Pharynx broadly oval, length less than one-half the diameter of oral sucker. Esophagus and intestinal ceca undeveloped but clearly outlined by large granular cells. These cells lie end to end and are constant in number. Esophagus bears a pair of lateral diverticula which extend antero-laterad from near the anterior end of the esophagus to level of pharynx. Esophagus has 5 cells; each diverticulum, 2; the ceca each have 14; and a large junction cell stands between esophagus and ceca; thus 38 cells form the gut anlage. Tissues between oral sucker and pharynx appear distinctly pink in living cercariae. Cystogenous glands such as occur in many cercariae were not observed. Eight dorsal cephalic ducts usually are visible anterior to the oral sucker and a number of less distinct additional ones are often seen in the same region. Excretory bladder with large posterior chamber and smaller anterior one, both contractile as in echinostomes. Entire excretory system echinostome-like (Fig. 2). Lateral siphons arise mesially from bladder, maintain small calibre and variable convolutions in passing to level of acetabulum where they enlarge abruptly and are thrown into 3 wide loops before reaching level of pharynx. Here they give off a single blunt lateral diverticulum then continue much reduced in calibre to level of oral sucker where they turn sharply dorso-mesiad and posteriorad to extend ventral to the gut as a narrow wavy canal unbranched to the posterior end. From here the smaller tubules and their exact relations with flame cells were not determined. Eight groups of 3 flame cells each were observed on either side of the body. The large excretory siphons contain very conspicuous spherical granules about 150 in number and fairly uniform in size. There are 17 or 18 flame-cell-like tufts distributed along the lumen of each main collecting tubule. Two small clumps of cells, one just posterior to acetabulum and one dorsal to the anterior border of acetabulum, represent anlage of reproductive organs. The tail is simple but powerful, attached somewhat dorsally just behind excretory pore. Caudal branch of excretory system extends to junction of first and second thirds of tail and branches into right and left forks. Both longitudinal and circular muscle bands extend to tip of tail.

REDIA AND MOTHER REDIA

(Figs. 11–14)

The rediae are located in the snail's kidney, gonad, and anterior part of the liver. Smaller rediae are colorless; older ones yellow, orange, or brown. All are fairly motile.

Description. Body sacculate to truncate. Collar near anterior end nearly always conspicuous and with 4 distinct lobes. Birth pore just behind dorsal lobe of collar. Posterior locomotor appendages located near junction of second and third thirds of body, may be long and motile or reduced to mere bulges in body wall. Pharynx well developed, terminal; gut simple, always extending far beyond collar and may reach posterior appendages in extended young rediae. Gut ventral, usually reaching about half-way between collar and appendages, and is well filled with black granules. Excretory system bilateral and separate on the two sides. Flame cells in

4 groups: 2 at level of appendages, the other 2 near the middle of body. Number of flame cells in each group equal, varying from 1 in very small rediae to 36 or more in largest ones. Excretory bladder on either side of body, half-way between flame cell groups, and joined to them by anterior and posterior excretory trunks.

One snail with a very old infection was opened on August 28. It contained very few mature cercariae in the tissues; very small rediae were more abundant than mature ones; and one of the larger rediae contained both cercariae and developing rediae (Fig. 13). This mother redia was less motile and more sacculate than the others. Posterior appendages were scarcely visible but the collar and birth pore were conspicuous. In the body of the mother redia were 2 fully developed cercariae, 2 well differentiated daughter rediae, and a number of embryonic forms ranging from small spheres to elongate bodies. The snail host had been collected 3 weeks previously and held in a shallow outside aquarium in running lake water. No accurate temperature readings were made but air temperatures had been sharply down for about one week when the snail was examined.

THE METACERCARIA

(Figs. 3, 10)

Metacercariae are normally found in the lateral line canal, and to a less extent under the scales, of fresh water fish. They also develop in the nostrils of tadpoles and fish, and occasionally may be found in the cloaca and associated ducts after prolonged exposure to heavy suspensions of cercariae.

After reaching the second intermediate host, the cercariae creep over its outer surface until an opening is found where they may enter without penetration of tissues. In the lateral line canal or other cavities they form a very thin-walled cyst which is always elongate. After a day or two, masses of cells surround the cyst and later form a loose capsule of connective tissue. Soon after encystment, further differentiation of a number of structures is initiated and in 7 to 10 days the metacercariae are infective. Within 3 days the acetabular spines are lost and body spines become visible. On about the fifth day a lumen begins to appear in parts of the digestive system and the excretory granules extend from the oral sucker to the bladder. After 2 or 3 days of further development the gut has a more or less complete lumen, body spines are well developed, and it is now infective. There is apparently very little if any growth, and the general arrangement of the digestive, excretory, and reproductive organs remains unchanged. Excysted metacercariae 12 days old measure 0.55 mm long when extended. Cysts of living metacercariae teased from the tissues measure around 0.32 mm by 0.21 mm. In stained balsam mounts the average is 0.23 mm by 0.15 mm.

THE ADULT

(Fig. 1)

The following description of *Psilostomum ondatrae* is based on a study of 8 paratypes (4 in toto and 4 sectioned) from U. S. N. M. Helm. Coll. No. 29750, 3 toto mounts received from Mr. E. C. Herber, 2 from Mr. L. R. Penner, and toto mounts and sectioned specimens from my own collection. Careful comparison of the specimens from the several sources leaves no doubt that they represent a single species, *Psilostomum ondatrae* Price, 1931. The material on which the original and only published description was based, apparently is not entirely typical of the species. The specimens were somewhat bloated by post mortem changes and had lost the entire cuticula in nearly all cases. Also they were flattened to such a degree that the normal proportions of the body were obscured.

Description. Body linguiform, flattened dorso-ventrally at the anterior end, more truncate in the post-testicular region. Length of mature specimens, 1.4 mm to 4.2 mm; greatest width at level of esophagus in unflattened condition; width equal to one-third the length. Entire surface beset with small triangular spines arranged in close alternate rows. Oral sucker subterminal, somewhat smaller than acetabulum. Ratio of the suckers changes in favor of acetabulum as growth progresses. The respective diameters of acetabulum and oral sucker in unflattened specimens 1.4 mm in length are 0.208 mm and 0.178 mm. In a flattened specimen 3.8 mm long, the diameters are 0.357 mm and 0.256 mm. Acetabulum located just anterior to middle of body in smaller mature worms but at the junction of the first and second thirds in the 3–4 mm class. From 10 to 17 oral ducts extend from anterior lip of oral sucker to region of pharynx. Prepharynx very short; pharynx oval, well developed; length equal to one-half the diameter of acetabulum. Esophagus short and with large esophageal diverticula on either side extending antero-laterad to the sides of pharynx. Intestinal ceca regular, extending to the posterior end of body. Excretory pore terminal; bladder truncate, bounded by posterior testis and intestinal ceca. Bladder bifurcates at its anterior end to receive large excretory trunks which lie on either side between the ceca and reproductive organs, and extend forward to the sides of acetabulum.

Testes tandem, cubiform to elongate transversely, located in the middle third of the posterior half of the body in worms not too much flattened. Their natural dimensions are slightly less than those of the acetabulum but due to relative lack of rigidity of the hind body, the least pressure causes remarkable alteration in the proportions of its contents. The testes may be enlarged in the frontal plane 3 or 4 times their normal dimensions. This is especially true of worms that have died in the gut. Cirrus pouch round, oval, pyriform, or truncate; usually located dorsal to acetabulum but may be slipped towards either side or entirely anterior to it; position in living worms shifts considerably with the different attitudes of the body but tends to remain more to the right side, and more towards the anterior slope of the acetabulum. It may reach the posterior border of the acetabulum, however. Cirrus sac nearly filled with seminal vesicle; cirrus simple, slender, and very long; prostate gland poorly developed. Genital pore located between acetabulum and bifurcation of gut.

Ovary half way between dorsal and ventral surfaces, anterior to testes and to left of median line of the body; circular to oval in outline; greatest diameter 90 μ in a 1.4 mm specimen and 215 μ in a slightly flattened specimen 3.8 mm long. Oviduct leads posterodorsad to the oötype which is surrounded by a well developed shell gland. Yolk ducts pass under intestinal ceca, turn abruptly dorsad and enter a voluminous yolk reservoir dorsal and posterior to shell gland. Seminal receptacle absent. Laurer's canal long, coiled irregularly. Posterior portion of uterus mostly ventral

to female reproductive complex and well filled with masses of spermatozoa, forming a receptaculum seminis uterinum. Loops of uterus few, mostly transverse, and intercecal. Metraterm on left side of cirrus sac, enters a shallow non-muscular genital sinus. Vitelline follicles large and closely distributed, forming a rather compact field on either side from level of pharynx to posterior end of body. The two sides are joined by a wide isthmus at the anterior end, and they converge at the posterior end to form a single post-testicular field ventral to the excretory bladder. Eggs regular in outline, oval, 80 μ to 90 μ long by 45 μ to 50 μ wide; operculum 15 μ to 18 μ in diameter; color pale brown.

Hosts and Pathology. Field hosts previously reported for this species are a muskrat (*Ondatra zibethica*) from Ontario, Canada (Price, 1931); a California gull (*Larus californicus*) from Klamath Falls, Oregon (Price, 1931), and young domestic chickens from Colorado (Newsom and Stout, 1933). In 1933, 56 specimens of *P. ondatrae* were taken by Mr. E. C. Herber from an osprey (*Pandion haliactus carolinensis*) near Carlisle, Pennsylvania, and in 1938 approximately 50 specimens were taken by Mr. L. R. Penner from each of 2 young Cooper's hawks (*Accipiter cooperi*) at the University of Michigan Biological Station on Douglas Lake, Michigan. The Cooper's hawks were taken from a nest and held in captivity throughout the summer. They were given a great diversity of foods including perch (*Perca flavescens*) and other species of fish from Douglas Lake. From my own experiments, laboratory infections have been obtained in the pigeon, chicken, duck, and canary.

In all of its bird hosts *P. ondatrae* was found in the proventriculus, each worm forming a deep pit in the mucosa. It was especially evident in the chickens and canaries that *P. ondatrae* is fairly pathogenic, each worm forming a separate lesion which is a deeply eroded pit with a raised orifice surrounded by a conspicuous reddish to purple area. Newsom and Stout (1933) reported epidemics of proventriculitis due to *P. ondatrae* in two different flocks of chickens in Colorado. Both flocks were young and ranged along the same drainage ditch where snails were abundant. Loss of appetite, malaise, and loss of weight preceded death which came "several days" following the loss of appetite. Death appeared to be due to starvation. Total losses in the two flocks were heavy. On gross examination the proventriculus was found to be greatly enlarged, with the mucosa, around the orifices of what appeared to be the glands, showing a deep reddening. In extreme cases a grayish exudate was observed on the surface. Microscopic examination showed a fibrinous exudate which was necrotic at the surface and heavily infiltrated with polymorphonuclear leucocytes in the deeper portion. The mucosa was quite edematous and held scattered polymorphonuclear leucocytes and monocytes. Small abscesses were observed in the lower portion of the mucous membrane. The worms had not invaded the glands. A similar epidemic caused by *Sphaeridiotrema globulus* in the gut of wild ducks has been reported by Price (1934).

There is some doubt about the location of the worms reported from the muskrat. Some of the type material was received in a container bearing on its label "from liver of muskrat" (Price, 1931). Since, however, it seems well established that the stomach (proventriculus) of both field and laboratory birds is a natural habitat for *P. ondatrae,* its occurrence in the liver of any animal is doubtful.

THE MIRACIDIUM

(Fig. 4)

Eggs were collected from the feces of an experimentally infected canary. From one to ten eggs were placed in small drops of clean water on a cover glass and sealed hanging in moist chamber slides. Development from day to day was then followed on several individual miracidia. Air temperatures during incubation ranged from 55 F to 80 F. After two weeks most of the miracidia were fully differentiated but some were fully twice the size of others. Hatching began to occur on the 16th day and all those that failed to leave the egg by the end of the fourth week died in the shell.

Fully developed miracidia are slightly longer than the egg and have the usual shape of echinostome miracidia. The cilia are distributed over the whole body and are of nearly equal length. The epidermal plates on which the cilia develop are in four transverse rows bearing 6, 6, 4, and 2 plates respectively from anterior to posterior. The posterior plates are dorsal and ventral rather than right and left, and each succeeding row is set to alternate with the last. The eye spots begin their development as a pair of circles containing scattered pigment which later concentrates in the mesial and posterior borders of the circle. In mature miracidia the pigment forms a broken crescent in each eye spot. Under low magnification this gives the appearance of an "X" at the junction of the first and second thirds of the body. A single pair of flame cells is differentiated early and may be seen in the posterior third of the body on about the tenth day. The flame cells gradually shift in relative position until the right is anterior to the middle of the body and ventral, while the left is somewhat more posterior and dorsal. This same condition has been observed by the writer (Beaver 1937) in the miracidia of *Echinoparyphium recurvatum* and *Echinostoma revolutum.* The proboscis is well set off from the rest of the body and is very active in mature miracidia. The gut is simple and extends to the level of the second row of plates.

EXPERIMENTS ON THE DETERMINATION OF HOSTS

Although he did not determine their location within the hosts, McMullen (1938) found that a few encysted metacercariae could be recovered from small bullheads (*Ameiurus*) by teasing apart all of the tissues after

exposure to large numbers of cercariae. When his experiments were repeated and similar results obtained, it was obvious that the small (5 cm to 7 cm) bullheads were not suitable hosts. If heavy suspensions of cercariae in small containers with a bullhead would produce no more than about a dozen metacercariae, field conditions could hardly be expected to provide any infective stages in this host. A study of the behavior of the cercariae led to the observation that they settle down and undergo extended migrations on a number of hosts including bullheads, tadpoles of several species, various scaly fish and salamanders. They appeared to enter all small orifices encountered and tended to proceed generally toward the head and sides of the animal. The nares became entirely filled with cysts. Upon entering the cloaca of fish and tadpoles, some of the cercariae encysted immediately, while others migrated up the mesonephric and Müllerian ducts. Cysts were found scattered along these ducts and in many instances several were found in the body cavity. Never more than a dozen or so would encyst in any one of these small hosts, however.

When a 5-inch perch was exposed to a heavy suspension of cercariae and the cercariae carefully observed through a hand lens, it was found that most of them settled down on the perch within a few minutes and some were observed entering the nares. Six hours later practically all cercariae had disappeared. At that time the perch was swimming crazily with its sense of orientation obviously restricted or disturbed. It was sacrificed and examined immediately. The lateral line canal was well filled with metacercariae and a number were observed under the adjacent scales. Subsequently an abundance of metacercariae were obtained by placing together in a larger tank, several infected snails along with perch (*Perca flavescens*), rock bass (*Ambloplites rupestris*), smallmouth black bass (*Micropterus dolomieu*), pumpkin seed (*Eupomotis gibbosus*), and bluegill (*Lepomis pallidus*). Field infections were found later in the lateral line canals of five-inch perch off Grapevine Point in Douglas Lake where the cercariae also occur. Nine individuals were examined and from 2 to 35 cysts were found in each lateral line canal.

When adequate material for experimental feedings to birds and mammals was obtained, it was already late in the summer and only 7 feeding experiments were carried to completion. Three White Leghorn chickens (an old hen and 2 young cocks) were fed perch carrying natural infections of *P. ondatrae*. The first was also given a perch and a sunfish each of which had about 100 metacercariae from 1 to 3 days old. On the 7th day after feeding, 3 mature worms were recovered from the proventriculus. Each of the other chickens was fed from 200 to 300 metacercariae that were 3 days old in addition to the naturally infected perch. After 6 days a single mature worm was taken from each of them. A duck was given metacercariae in small bullheads (*Ameiurus*) that were exposed to

cercariae 10 days previously. After 9 days a single mature worm was found in the proventriculus. A pigeon was fed 3 small bullheads which were carrying about 10 metacercariae each. The metacercariae were 7 days old. After 3 days a single immature worm was recovered from the proventriculus. A canary was fed 35 metacercariae in a few of the scales from a rock bass (lateral line canal) that had been exposed to cercariae 8 days previously. On the 7th day eggs were abundant in the feces and the next day 5 mature worms were taken from the proventriculus. About 100 six-day-old metacercariae in the lateral line canal of a rock bass were fed to an albino rat. It was negative when examined on the sixth day.

It would appear that metacercariae are not infective until at least 7 days of age and the time required for reaching maturity in the final host is 6 or 7 days. Although other parts were carefully examined, the proventriculus was the only site of infection discovered in any of the hosts. With special attention to the proventriculus, the following birds, all of which were known to feed where the cercariae of *P. ondatrae* are found, were examined: 2 Caspian terns, 6 herring gulls, 1 American merganser, 1 kingfisher, and 1 crow. All were negative for this species.

SUMMARY OF LIFE HISTORY

In the adult stage *P. ondatrae* parasitizes fish-eating birds such as ospreys and gulls and also may occur in muskrats. It is located deep in the mucosa of the proventriculus. The eggs are large, have heavy shells, and are undeveloped when laid. After about eighteen days at summer temperatures the miracidia hatch, become free-swimming, and enter the primary intermediate host (*Helisoma antrosum percarinatum*). The developmental stages leading up to the rediae have not been determined but it is known that the rediae generate both second generation rediae and cercariae, and both may be found in the same individual. The cercariae leave the snail during the night and find their way into the lateral line canal, into the nares, and to a less extent under the scales of freshwater fish. Here a thin-walled cyst is formed and slight further differentiation in the body of the metacercaria takes place. After a week to ten days the metacercariae are infective and maturity is reached on the sixth day in experimental final hosts.

DISCUSSION

Life histories are known for two other species of the PSILOSTOMIDAE. Rediae, cercariae, metacercariae, hosts, and the mode of transmission from host to host have been described for *Psilotrema spiculigerum* (Muhling) by Mathias (1925) and for *Sphaeridiotrema globulus* (Rud.) by Szidat (1937). The miracidium of *Psilotrema spiculigerum* also has been described. In all three species the rediae are echinostome-like with a well developed pharynx and collar, a single pair of appendages near the poste-

rior end, four groups of numerous flame cells, and with heavy pigmentation in the older stages. The snail host for *Psilotrema spiculigerum* and *S. globulus* is a small operculate species, *Bythinia tentaculata* while for *Psilostomum ondatrae* it is a pulmonate species, *Helisoma antrosum percarinatum*. In the character of the excretory system, suckers, cuticula, tail, and swimming behavior the cercariae of the three species are essentially similar and echinostome-like. *Psilotrema spiculigerum* encysts in the open, either on the snail host's shell or on submerged vegetation while *Sphaeridiotrema globulus,* like many echinostomes, uses the same snail as both primary and secondary intermediate hosts. After a period of swimming, it returns to encyst on the inner side of the shell and between the mantle and shell of *Bythinia tentaculata*. In both species the cyst wall is heavy. There is considerable contrast in the above with the habit observed in *Psilostomum ondatrae* where the metacercariae are found in thin-walled elongate cysts in the lateral line canal of fish. It is not definitely known whether like *P. ondatrae,* the other two species require several days in the metacercarial stage or, like some of the echinostomes, they are infective soon after encystment. Each of the three species reaches maturity after about one week in the final host and the ova are large and entirely undeveloped when expelled. The miracidia of *Psilostomum ondatrae* and *Psilotrema spiculigerum* are similar in their development and structure. In the character of their epithelial plates, eye spots, excretory system, size, and contours, they resemble the miracidia of *Echinostoma, Echinoparyphium, Hypoderaeum* and other echinostomes. It is apparent that the psilostome life cycle in each of its stages demonstrates close relationship with the ECHINOSTOMIDAE.

SUMMARY

1. *Cercaria thomasi* McMullen, 1938, is redescribed and experimentally demonstrated to be the cercaria of *Psilostomum ondatrae* Price, 1931, which also is redescribed.

2. The redia, mother redia, and miracidium are described and shown to be echinostome-like in their morphology.

3. The lateral line canal of several species of fish is the most common site of metacercarial encystment.

4. Four laboratory hosts (the chicken, duck, pigeon, and canary) and two new field hosts (the osprey and Cooper's hawk) are reported for the adult.

5. *P. ondatrae* is relatively pathogenic, producing restricted erosion and ulceration in the proventricular mucosa.

6. All of the known psilostome life histories resemble each other very closely and are essentially echinostome-like in the character of the larval stages.

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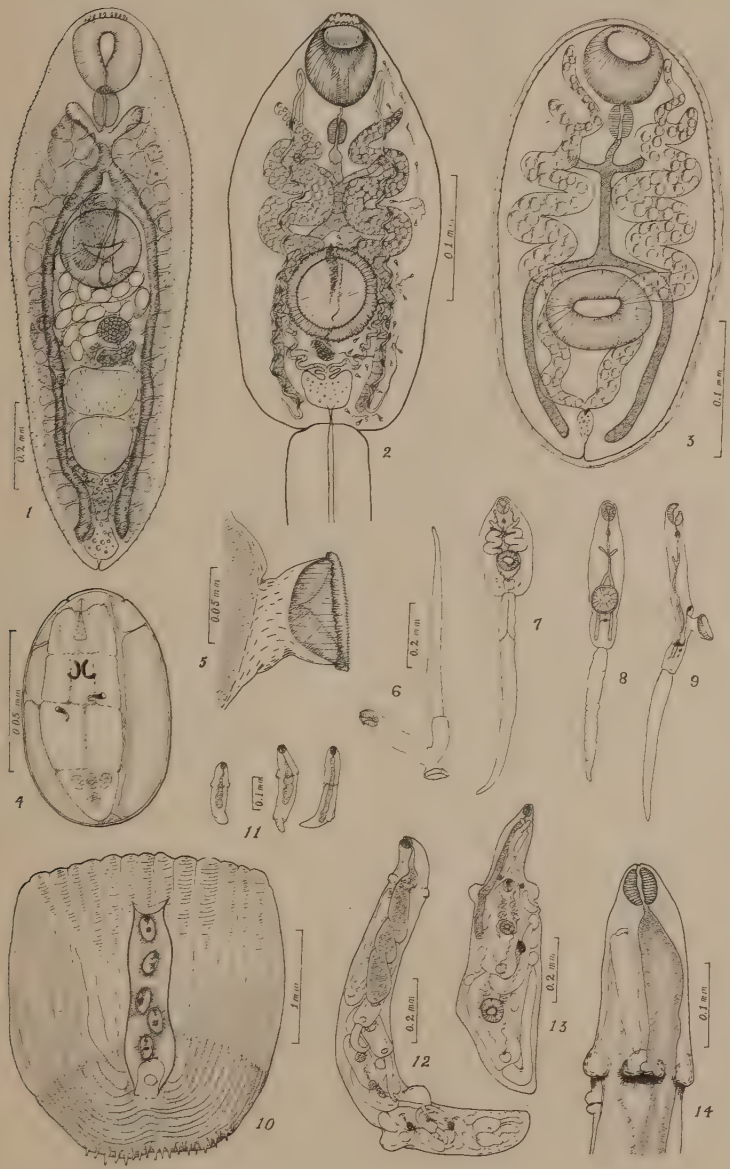
Explanation of Plate

Drawings made with the aid of a camera lucida.

1. Young adult from experimental chicken. Ventral view.
2. Cercaria. Ventral view of body unflattened.
3. Metacercaria from the lateral line canal of *Perca flavescens*. Age 12 days.
4. Miracidium after 15 days incubation.
5. Acetabulum of cercaria, protruded. Lateral view.
6, 7, 8, and 9. Cercariae showing typical attitudes of the body and tail.
10. Metacercariae in lateral line canal under a scale removed from *Ambloplites rupestris*.
11. Three young rediae showing collar, appendages, and gut.
12. Redia. Ventral view of mature specimen of average size, moderately contracted.
13. Mother redia. Lateral view of contracted specimen under slight pressure of cover glass.
14. Redia. Lateral view of anterior end.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PARASITIC LESIONS IN TURTLES*

ELSIE WIECZOROWSKI

Parasites, apparently largely because of the toxic substances which they secrete, have been recognized as one of the factors which may excite the formation of neoplasms. Borrel (1) found nematodes in the center of sarcomas and adenocarcinomas of rats. In 1911, Haaland (2) described tumors which occurred in the mammary glands of mice and apparently were caused by nematode parasites which were found within the tumor. Papillomatous gastric tumors containing a species of *Dispharagus* were reported by Wasielewski (3). A possible relationship between trichiniasis and cancer has been studied by many investigators. Ewing (4) mentions finding trichina in cancerous tongues quite frequently. The work of Fibiger (5) on the experimental production of cancer in rat stomachs is well known. In rats fed with cockroaches, infected with the nematode, *Spiroptera* sp., chronic inflammation occurred followed by the formation of papillomatous carcinomas which eventually metastasized. In 1916, Izumi (6) reported four cases in man in which ascaris had invaded the pancreas by way of the pancreatic duct. Egg nodules and larger lesions, associated with the entire worm, were described in detail.

Most of these papers discuss cases in which the end result of parasitic infection was a malignant neoplasm occurring in lower animals. The following report discusses two non-malignant pathological changes found in the pancreas and other organs of twenty turtles of the species *Chrysemys marginata* and *Chrysemys elegans* purchased from a commercial frog farm in Wisconsin.

The turtles were killed in May, June, August, October and December. Parasites found in the intestine were preserved for further investigation. The pancreas, with the portion of the intestine adjacent to it, as well as portions of the liver, lung, bladder and spleen were fixed in Zenker's solution. All of the tissues were sectioned from six to ten micra thick and stained in Delafield's hematoxylin with eosin counterstain.

With the exception of ten specimens of a nematode, *Spiroxys contorta* and two small immature trematodes which closely resembled *Telorchis nematoides* (7), the hundreds of parasites removed from the intestines of the turtles were of the acanthocephalan *Neoechinorhynchus emydis*, which occurred in large numbers in those turtles which were infected, and the nematode *Camallanus trispinosus*, which infected a larger per-

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centage of the turtles but was less numerous than the acanthocephalan (8).

MORBID ANATOMY

Two types of lesion were distinguished grossly and microscopically. Large lesions, which were brown to blackish brown in color and varied in size from one to three millimeters, were always located in the vicinity of the main pancreatic duct. Macroscopically the smaller lesions appeared as tiny golden-brown specks scattered throughout the pancreas, the remainder of which was creamy yellow and of rather firm consistency typical of the normal gland.

MICROSCOPIC ANATOMY

Microscopic examination of the smaller lesions disclosed a typical foreign body reaction on the part of the tissue surrounding parasite eggs which were very large, measuring from 0.093 to 0.141 mm in length and from 0.082 to 0.090 mm in width. The moderately thick, amber colored shells were usually badly cracked and disfigured in the sections. The eggs were not those of any of the species found in the digestive tract of the turtles. They resembled the eggs of *Proparorchis artericola* (9) in size and appearance.

In typical nodules, the eggs were surrounded by foreign body giant cells outside of which there was a very small amount of fibrous tissue (Fig. 3). In many cases, small blood vessels or capillaries led directly to the focus of the eggs. In such instances, red blood cells were often seen within or just outside of the capsule (Fig. 4).

Sections of other organs disclosed a wide distribution of lesions similar to those just described. Such egg nodules were found in the vascular portion of the lung, the mucosa and submucosa of the duodenum, the tunica propria of the urinary bladder, the interlobular triads of the liver and the Malpighian corpuscles of the spleen. In the latter, the nodules were more numerous than in any other organ including the pancreas.

The large lesions, which were located in the vicinity of the main pancreatic duct, consisted of a central area of necrosis surrounded by giant cells in some of the lesions. Peripheral to this there was a zone of heavy lymphocytic infiltration with a scattering of leucocytes in which the proportion of eosinophils could not be accounted for by an increase of these cells in the blood. The lesion was encapsulated by a rather dense layer of fibrous tissue which formed a definite line of demarcation between the pathological and normal tissue (Fig. 1). Some of these lesions were very extensive so that only small portions of normal tissue could be observed in cross-sections of the entire gland (Fig. 2).

Only four of the large lesions described above presented actual evidence of parasitic etiology. Serial sections of these lesions revealed, in one, a worm which closely resembled *Telorchis nematoides*, in another, a specimen of *Camallanus trispinosus*. The operculated eggs of *T. nematoides* were in the center of another lesion and the fourth contained unidentified ascaris-like ova.

Characteristic changes in the pancreatic ducts were marked subepithelial lymphocytic infiltration and an extensive proliferation of fibrous tissue. A very small trematode was seen in the wall of one of the ducts just below the epithelium which had undergone pronounced desquamation. The worm was surrounded by a heavy infiltration of lymphocytes.

DISCUSSION

The large lesions described in the foregoing paragraphs appear to be due to invasion of the pancreatic duct and, eventually, the pancreatic tissue by parasites from the intestine. This seems to be the logical etiology of the lesions because of their constant association with the duct and absence from other parts of the gland, the presence of the eggs of the intestinal fluke *Telorchis nematoides*, in one of the lesions and the resemblance of the fluke in another lesion to this species. Coming to rest in the wall of the duct or the glandular tissue, the irritating presence of the living worms plus, perhaps, the toxic substances which they secrete arouses an inflammatory reaction on the part of the tissue. The resulting lesions resemble infectious granulomas in their microscopic appearance and inflammatory character and show no malignant characteristics. It may be that the more extensive lesions are due to the additional presence of bacteria carried from the intestine by the parasitic worms.

The egg nodules differ from the granulomas discussed above in type of tissue reaction, distribution and avenue of infection. These lesions are typical of the reaction of any tissue to the presence of small foreign bodies. Because of their wide distribution in so many organs of the body it is apparent that the avenue of infection is not by way of the pancreatic duct. The close relationship of the nodules to the splenic corpuscles and to capillaries and small blood vessels of other organs seems to indicate that the eggs are carried to their various foci by the blood. The eggs of *Proparorchis arcticola* are also generally distributed. Ward lists *Chrysemys marginata* as one of the hosts of this parasite.

CONCLUSIONS

1. Parasitic lesions were found in the organs of twenty turtles of the species *Chrysemys marginata* and *Chrysemys elegans*.

2. Large parasite eggs, apparently distributed by the blood, were

found in the spleen, pancreas, intestine, lung, liver and bladder where typical foreign body reactions resulted in nodule formation.

3. Larger lesions, located in the vicinity of the pancreatic ducts, probably were due to parasites from the intestine which invaded the walls of the ducts and the pancreatic tissue. The inflammatory reactions to the irritating presence of the living worms and the microscopic appearance of the lesions are typical of the infectious granulomas.

4. The eggs correspond in size and appearance to those of *Proparorchis artericola*. The fluke in the larger lesion resembles *Telorchis nematoides*.

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Explanation of Plate

Fig. 1. Portion of a large lesion of the pancreas containing a small immature trematode. Camera lucida drawing.

Fig. 2. Section showing extent of large lesion, a portion of which is drawn in detail in Fig. 1.

Figs. 3 and 4. Egg nodules in the pancreas. Camera lucida drawings.

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OBSERVATIONS ON *BALANTIDIUM COLI* FROM SWINE IN CULTURE

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Most of the data here presented were derived incidental to an attempt to obtain bacteria-free cultures of *Balantidium coli*. Glaser and Coria (1935) effected a partial purification of this protozoon by allowing it to migrate through semi-solid agar but were unable to eliminate all the bacteria by this method. It was, therefore, determined to pick out and wash single organisms with a micropipette and attempt to initiate cultures from them. This method was used with success by Barker (1935) and Rees (1937) for the bacteria-free cultivation of marine dinoflagellates and *Trichomonas foetus*, respectively.

All cultures were made in the liquid medium described by Glaser and Coria (1935), which is composed of 5 per cent horse serum in Ringer's solution, to which a little sterile rice starch is added. Single balantidia growing in this culture medium were isolated with a Barber type micropipette and transferred to fresh culture tubes. The tubes were incubated at 37° C, and observations were made at intervals ranging from one or two days to two weeks. In all, 76 balantidia were thus isolated and treated. In no case were living protozoa seen on subsequent observations. This result is confirmatory of that reported by Knauff (1935), who stated that numerous attempts to isolate single balantidia and obtain pure lines were unsuccessful.

It was then decided to determine the number of balantidia necessary to initiate a successful subculture in the presence of contaminating bacteria. In this investigation, a single strain of *Balantidium coli* (strain No. 1111) obtained from the cecum of a pig was used. At the start of the experiment this strain had passed through 52 serial transfers in the Ringer's-serum-starch medium. Balantidia from many culture tubes were pooled and mixed thoroughly. One-tenth cubic centimeter was then withdrawn with a pipette and fixed in 0.9 cc of a mixture of 1 part formalin and 4 parts Gram's iodine solution. After thorough mixing, a 0.05 cc sample was removed and the balantidia in it counted. By multiplying the resultant number by 200, the number of balantidia per cc in the pooled sample was obtained. The latter was then diluted to 10,000 balantidia per cc and inoculations of fresh tubes were made from it.

After several preliminary series had been run to determine the approximate number of balantidia necessary to obtain growth, 50 tubes each

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were inoculated with 1,000, 2,000, 3,000, 4,000, 5,000, and 10,000 balantidia. Not all the tubes were inoculated on the same day, since it was not possible to obtain sufficient numbers of *Balantidium*. Usually not over ten tubes of any one inoculum size were prepared on the same day, but several different inocula were used. For example, if 50 tubes were set up, ten each would be inoculated with 1,000, 2,000, 3,000, 4,000, and 5,000 balantidia, respectively.

The culture tubes were incubated at 37° C, and observations were made after 48 hours, since it had been observed that the protozoa of this strain would not multiply to any degree after this period. The results were recorded in five categories: –, no *Balantidium* seen; +, a very few *Balantidium* present per drop taken from the bottom of the tube; ++, few *Balantidium* present (1–3 per field under the low power of the microscope); +++, moderate numbers of *Balantidium* present; and ++++, many *Balantidium* present. In the last category all the rice starch had been utilized. Further subcultures could be made only from the +++ and ++++ tubes, and only from the latter with ease. The results are recorded in Table 1.

TABLE 1.—*The relation of numbers of* Balantidium coli *in the inoculum to the character of the resultant culture*

Number of Balantidium in inoculum	Results (number of tubes)				
	–	+	++	+++	++++
1,000 ...	32	2	14	2	0
2,000 ...	9	4	12	23	2
3,000 ...	1	1	7	27	14
4,000 ...	0	1	8	16	25
5,000 ...	0	0	4	13	33
10,000 ...	0	0	0	4	46

Key: The –, +, ++, +++, ++++ columns indicate the relative abundance of balantidia found in the designated number of cultures.

An inoculum of 1,000 *Balantidium* gave negative results in 64 per cent of the tubes and ++++ cultures in none. An inoculum of 2,000 *Balantidium* gave negative results in 18 per cent and ++++ cultures in 4 per cent of the tubes. The other tubes gave cultures intermediate in character. An inoculum of 3,000 balantidia gave 2 per cent negative and 28 per cent ++++ cultures. Tubes inoculated with 4,000 or more balantidia were never negative. An inoculum of 4,000 gave 50 per cent ++++ cultures, while 5,000 and 10,000 balantidia gave 66 per cent and 92 per cent good cultures, respectively. Therefore, in order to achieve consistently favorable results in subculturing these balantidia, an inoculum of at least 10,000 individuals is necessary, and a larger one would be preferable.

One explanation for the large number of balantidia necessary to initiate successful subcultures is that the fission rate of the protozoa is so slow in comparison to that of the bacteria present that the latter multiply rapidly and use up some necessary constituent of the medium and/or release some inhibiting or toxic substance before the multiplication of the

balantidia has progressed far enough to allow them to compete successfully. In this case, the protozoa would survive only if sufficient numbers were present at the start. The results could also be explained as an example of the "allelocatalysis" of Robertson (1924), which he defines as "the acceleration of multiplication by the contiguity of a second organism in a restricted volume of nutrient medium."

As stated above, these results were obtained with *Balantidium coli* strain No. 1111, which had been habituated to the artificial culture medium employed. It is probable that other strains of *Balantidium*, and even this strain under other environmental conditions, might give varying results. In different culture strains in the same culture medium the numbers and species of bacteria and other microorganisms present as contaminants may influence the development of the cultures. The morphological differences between the *coli* and *suis* types, which may be accompanied by physiological differences, may also be a factor. It has not been definitely proved that these two types are distinct species.

DISCUSSION

Attempts to obtain viable cultures of *Balantidium coli* from the cecal contents of swine have met with highly variable results. Cultures can be initiated and propagated from some pigs with ease, while balantidia from other pigs when placed in culture tubes fail to produce viable cultures. A prime factor is the number of balantidia used to initiate the culture. As has been shown, a rather large number of protozoa is essential. The physiological character of the balantidia and the types of concomitant organisms present may also be important. However, almost nothing is known of the influence of these factors.

Pseudomonas aeruginosa (*Bacterium pyocyaneum*) has been reported to prevent successful cultivation of *Trichomonas* (Hogue 1928). In the case of *Balantidium*, however, it apparently does not have the same effect. One strain of *Balantidium* (No. 3724) which contained *Pseudomonas aeruginosa* was carried through 42 serial passages over a period of about 3 months in Ringer's-serum-starch medium, when it died out for some unknown reason. No special precautions were taken in carrying it on. It was observed that the pyocyanin produced by the bacteria, which is the agent responsible for their toxic action on *Trichomonas*, was present only in the reduced form in the greater part of the culture tubes. The characteristic green color was visible only near the surface. However, when fluid from the bottom of a tube where the *Balantidium* congregated, was removed with a pipette and exposed to air, the greenish color of pyocyanin quickly developed.

Blastocystis is very detrimental to the successful cultivation of most intestinal protozoa. However, a strain of *Balantidium* (No. 14377) has been cultivated in the presence of multitudes of *Blastocystis* for 52 serial

transfers over a period of about three and a half months without any diminution in numbers or vitality. Apparently, therefore, neither *Pseudomonas aeruginosa* nor *Blastocystis* necessarily prevents the propagation in vitro of *Balantidium coli*.

SUMMARY

1. Failure of 76 attempts to cultivate *Balantidium coli* of swine from single organisms isolated by micropipette is reported.

2. It has been shown for a strain of *Balantidium coli* cultured in Ringer's-serum-starch medium, that an inoculum of at least 10,000 individuals is necessary for consistently successful subculture.

3. *Balantidium coli* from swine has been cultured successfully in the presence of *Pseudomonas aeruginosa* and of *Blastocystis*.

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DIFFERENTIAL BLOOD PICTURE AND TOTAL CELL COUNT ON NORMAL AND TRICHINA INFECTED ALBINO RATS*

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Studies on human trichinosis have shown that eosinophilia is so constantly associated with trichinosis that its presence may serve as an important factor in the diagnosis of the disease. Considerable variation occurs in the degree of eosinophilia and the length of time it persists. There is a pronounced leucocytosis and eosinophilia in human infections. Cases with no eosinophilia but with a general leucocytosis are cited by Conner (1929) as an atypical form of trichinosis. Although the haematologic response of man to infections with *Trichinella spiralis* is quite well established, the same cannot be said of a number of laboratory animals that are used a great deal in experiments on this parasite.

Maass (1933) made blood counts on trichina infected hogs. Of nine animals with normal blood pictures, two each were fed 100, 200, 1,000, and 2,000 encapsulated trichinae from muscle; the ninth pig served as a control. Eosinophiles increased gradually beginning a few days after infection; reached a maximum in 20 to 24 days (that is when the young trichinae penetrated the muscle) constituting 10, 12.5, 20, and 27 per cent, respectively; then fell rapidly, and gradually returned to normal within two or three days. Eosinophilia in the control animal varied irregularly from 0.5 to 4.5 per cent. At autopsy all the hogs that had been infected showed trichinae, particularly in the diaphragm, and also in the cheek, thoracic and the thigh muscles. The eosinophilia described by Maass was accompanied, in every case, by a leucocytosis in proportion to the number of worms ingested.

In monkeys McCoy (1932) was unable to demonstrate a marked eosinophilia in his experimental work.

Wantland (1937) studied the blood in normal and trichina infected white rabbits and found that variations occur even in normal animals, but in rabbits infected with 5,000 to 10,000 larvae a leucocytosis developed from the second week after infection. The animals that showed the severest symptoms also showed a greater eosinophilia; however he concluded that the percentage of eosinophiles was not an index of the severity of the infection.

Van Someren (1938) in a series of differential counts on rats, both

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uninfected and infected with trichinae, found that infected animals that had received 400–600 larvae developed a relative neutrophilia with a corresponding lymphocytopenia within 2–4 days. This became less marked as the infection progressed, though the percentage of neutrophiles remained above, and that of the lymphocytes below, the normal average. He also found a slight eosinophilia, which reached its maximum 7–16 days after the infection.

No total leucocyte counts were made.

The present investigation is a study of the effect of trichina infection on the blood of albino rats.

TECHNIQUE

A series of twenty albino rats was used in these experiments. Total and differential blood counts were made at two-day intervals for a period of two weeks preceding the infections. The counts were made on blood taken from the heart and the tip of the tail alternately, in order to circumvent the error due to local inflammation. By cardiac punctures it was possible to obtain blood in sufficient quantities to make oxalation possible, and thus it was easier to make counts. The animals were kept under deep anesthesia when obtaining blood.

Total counts were made with a bright-line Neubauer haemacytometer. Blood smears were made on a coverslip and stained with Wright's stain. Differential counts were made by the 4-field meander method, beginning at the margin of the smear, working toward the center, then working back to the edge, alternately. After 25 leucocytes had been counted, a shift was made to another corner of the smear, until 100 cells had been counted.

BLOOD COUNTS ON UNINFECTED RATS

Blood counts were made on uninfected rats for a period of two weeks to determine the normal blood picture. Six counts were made on each of these 20 animals at 48-hour intervals. The normal ranges of red, white and differential counts in the rats used in these experiments were as follows:

Total red cells: 6,800,000–9,200,000, average 7,500,000.
Total white cells: 13,000–16,000, average 15,000.

	Range	*Average*
Neutrophiles	16–25 per cent	18.0 per cent.
Lymphocytes	70–83 " "	76.0 " "
Monocytes	0–4 " "	1.0 " "
Eosinophiles	1–3 " "	2.0 " "
Basophiles	0–2 " "	0.5 " "

The neutrophiles and lymphocytes are in inverse proportion to the corresponding percentages in man given by Van Someren (1938). The percentages of neutrophiles and lymphocytes are approximately the same

in swine as in albino rats according to Maass (1933). The total white cell counts showed daily fluctuations from 200–700 cells per cmm. The neutrophiles and lymphocytes in the differential counts of individual animals varied from 3–5 and 4–10 per cent respectively.

BLOOD COUNTS ON INFECTED RATS

To infect the rats skeletal muscles of heavily infected rabbits were removed and ground by a "fine-knife" meat grinder. The ground-up meat was thoroughly mixed and samples counted for worms by the pressed-preparation method and determined as the number per gram of muscle. This is believed to be a more accurate method for infection than using worms freed by artificial digestion. (We found that many trichina larvae when subjected to the action of pepsin for six to eight hours after being freed of their cysts were immobilized and others partially digested—obviously injured or killed.)

Four rats were given 25–50 trichina larvae, two 100–200, one 400–500, eight 600–700, three 900–1,000 and two 1,200–1,300.

The four rats fed 25–50 larvae showed very little change in the total leucocyte counts, which did not exceed 17,000, nor did eosinophiles exceed 3 per cent at any time. The neutrophiles increased from 18 to 30 per cent in one; 20 to 29 per cent in another; remained 25 per cent in another and rose from 24 to 33 per cent in the last. The lymphocytes decreased slightly, from 70 to 68 per cent, 80 to 67 per cent, 74 to 72 per cent, and 74 to 66 per cent respectively. These results are based on 100 cell counts.

In the two rats given 100–200 larvae there again was very little change in the total leucocyte counts, which did not exceed 18,500. The eosinophiles ranged between 1 and 3 per cent during the course of the infection. The neutrophiles increased in number, in one animal from 20 to 37 per cent, and in the other from 18 to 39 per cent. The lymphocytes decreased from 76 to 60 per cent and from 79 to 57 per cent respectively.

In the rat given 400–500 larvae there was an increase in the total white cell count, reaching 25,000 the 16th day after infection. Thereafter it declined until it again reached its normal range. The eosinophiles in this animal did not exceed 2 per cent. The neutrophiles increased from 18 to 48 per cent, while the lymphocytes decreased from 79 to 49 per cent.

In the eight rats fed 600–700 larvae, the total leucocyte counts went to 33,000 and returned to within the normal range of 13,000 to 16,000 after several weeks. The eosinophiles remained very few in number, although in two rats they reached 7 and 8 per cent on the 13th and 14th days, but dropped back to 2–3 per cent within one or two days thereafter. In the other animals the eosinophiles never exceeded 3 per cent. The neu-

trophiles increased from an average of 18 per cent to an average of 55 per cent. The lymphocytes decreased from 76 to 40 per cent on the average. Fig. 1 shows a graph of the typical leucocyte count in a rat

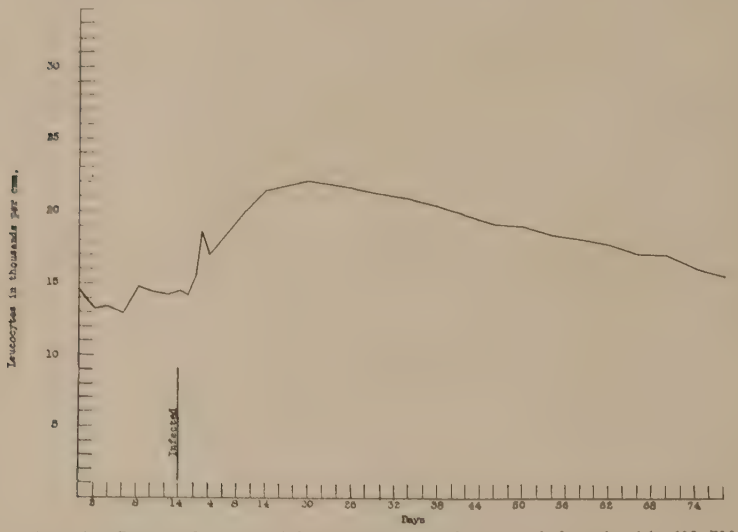

FIG. 1. Graph of the total leucocyte counts in a rat infected with 600–700 trichinae from muscle. The erythrocyte count underwent no changes during the course of the infection.

belonging to this group, in which total erythrocyte and leucocyte counts were within the normal limits during the 15 days previous to infection. After infection the erythrocytes remained about the same but the leucocyte count increased steadily during the time that the larvae were developing and mating in the small intestine. Increase continued until it reached its peak (23,000) on the 18th day after infection, with a gradual diminution for several weeks to the normal range.

The differential counts, as represented by the graph, Fig. 2, were within the normal limits before infection, but within 24 hours after feeding a marked change took place in the lymphocyte-neutrophile ratio. The percentage of neutrophiles increased and the lymphocytes decreased. This continued for 48 hours, the time during which the larvae develop into adults (Beahm, 1939, and others) and produce a very irritating effect on the digestive tract. The intestines become haemorrhagic, the Peyer's patches are very much enlarged, and the entire enteric tract, below the stomach, is distended with an enormous amount of mucus. It is this period in the parasite's life-cycle that is the most dangerous for the host, and it is during this time that most of the deaths occur.

At 72 hours a noticeable change usually begins to take place, neu-

trophiles are beginning to decrease and lymphocytes to increase. The blood counts now begin gradually to return to the normal range. At this stage the eggs are fertilized and develop; the young larvae are produced, and begin leaving the mother on about the seventh day. At this time they begin migrating through the body of the host. This migration takes place for considerable time, coming to an end approximately 17 days after infection (Beahm, 1939, and others).

The three rats given 900–1,000 larvae died after 28–35 hours, before

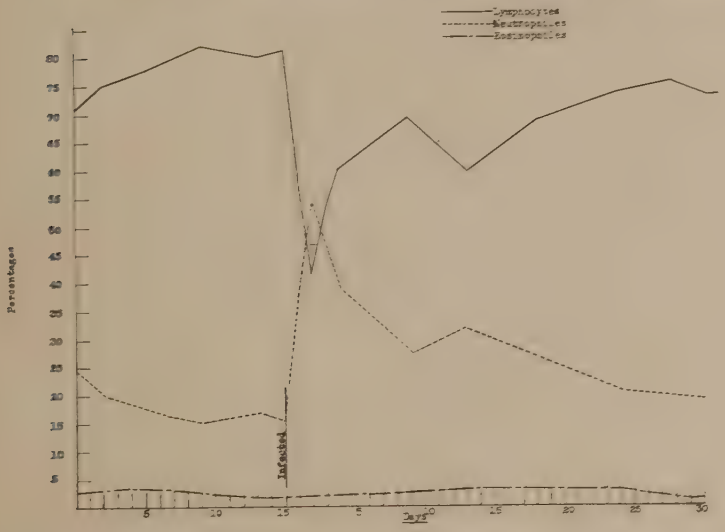

FIG. 2. Differential count of rat infected with 600–700 trichinae from muscle. The eosinophiles, basophiles, and monocytes underwent no changes during infection.

there was an increase in the total leucocyte count. The eosinophiles did not increase in number, but the neutrophile count which ranged from 17 to 19 per cent before infection rose to 62–65 per cent within 24 hours, and the lymphocyte count dropped from 75–80 per cent to 31–34 per cent.

The two rats fed 1,200–1,300 larvae died 30 hours after infection. The total white count did not undergo an increase before the animals died. The eosinophile count did not exceed 3 per cent. The neutrophiles rose from 18 and 20 per cent to 58 and 62 per cent respectively. The lymphocytes dropped from 74 and 72 per cent to 36 and 33 per cent.

DISCUSSION

The total erythrocyte count was unaltered as a result of trichina infections in these rats. The total leucocyte count underwent great changes, depending upon the severity of the infection. If animals were given 600–

700 larvae, there resulted a gradual increase from the normal count of approximately 14,000–15,000 white blood cells per cmm to as much as 33,000 two weeks after infection. If, on the other hand, an animal were given only 25–50 larvae the count changed very little. Doses between these two extremes brought about reactions which were in direct proportion to the severity of the infection. The same factor was influential in controlling the differential count.

There appears at first a relative and then an absolute neutrophilia, bringing about a relative lymphopenia. Within 3 or 4 days the lymphocytes begin making a noticeable increase, and there results a lymphocytosis and a relative neutropenia. The relative neutropenia is not necessarily due to the fact that neutrophiles have begun to decrease, but probably results from the entrance of many lymphocytes into the blood stream. This seems reasonable because there is an actual increase in the total leucocytes, as demonstrated by the total white cell count.

The eosinophile count, which runs very low in normal animals, made no appreciable change after infection. Maass (1933) found that an eosinophilia accompanies trichina infections in pigs. Wantland (1937) claims the same for rabbits. Van Someren (1938), in his report, stated that rats given 600 trichinae showed maxima of 20 per cent eosinophiles on the 16th day, and 13 per cent on the 11th day. In no case were we able to demonstrate more than 7–8 per cent eosinophiles, and we were only able to find them in this number in two out of twenty rats. These counts of 7 and 8 per cent eosinophiles appeared on the 13th and 14th days respectively. In both of these cases the eosinophile counts dropped back to 2–3 per cent within one or two days. In the other 18 animals these cells never exceeded 3 per cent. Van Someren made all of his counts from tail punctures, and he stated in his report that his rats showed a low-grade eosinophilia of about 4–9 per cent before infection. His explanation for this was that a number of them were infected with the scab mite, *Notoedras muris,* especially on the tail.

There was no change in the basophile or monocyte counts after infection.

SUMMARY

Blood counts, both total and differential, were made on a series of 20 albino rats. They showed that the total erythrocytes were not altered following infection with *Trichinella spiralis.* The total leucocytes increased, being approximately doubled after doses of 600–700 trichinae larvae; however if only 25–50 larvae were ingested the count showed practically no change although at autopsy these animals were seen to be infected. Doses between these two limits brought about reactions somewhat proportional to the severity of the infection.

The differential count was also dependent upon the number of tri-

chinae ingested. If the infection was severe enough a relative and absolute neutrophilia appeared. This took place at the expense of the lymphocytes, thus bringing about a relative lymphopenia. Within 3 or 4 days the lymphocytes began to increase noticeably and a lymphocytosis resulted. The relative decrease in neutrophiles was due to the increased lymphocytes.

The eosinophile count, which was very low in normal rats, showed no appreciable change after infection; the same was true of the basophile and monocyte determinations. In so far as these studies go they indicate that the eosinophilia, so characteristic of trichina infections in man and swine, is not elicited in the rat.

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OPISODASYS JORDAN 1933, A GENUS OF SIPHONAPTERA[1]

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The flea genus *Opisodasys* Jordan (1933) contains 5 known species, all from North America. Some of the existing descriptions are inadequate and for two species only one sex has been described. In order to facilitate the identification of the species of this genus, supplementary descriptions, drawings[2] of critical characters, and the missing sex descriptions are presented.

Opisodasys enoplus (Rothschild) 1909

This species was described from a single male taken from a California grey squirrel at King's River, California. No other record of its occurrence is available and the female has not been described.

Female

Head: Frontal tubercle very small, antennal groove extending 2/3 depth of head, bordered dorsally with fine setae, most numerous posteriorly. Long bristles on 2nd segment of antennae not equaling club. Eye well-pigmented, gena broad. Genal row of 4 bristles on one side and 3 on the other (this same irregularity occurs in a male of this species). Bristles in occipital row, all short, except 2nd ventral one which extends to mesothorax. Rostrum equaling trochanter.

Thorax: Pronotal comb of about 24 spines. One lateral bristle on fore femur. First pair of plantar bristles displaced ventrally on all tarsi. Metanotum with an apical spinelet on each side.

Abdomen: Apical spinelets on each side of tergites I to IV, 3–3–2–1 respectively. Each side of tergites II to VII with an anterior row of 4–6 short bristles and median row of 6–7 long bristles, alternating with very fine setae. Each side of sternites II to VI with 3–5–4–4 and 4 bristles respectively.

Modified segments: Sternite VII with short rounded posterior dorsal lobe, and about 18 bristles irregularly arranged as figured. Three lateral bristles on anal tergite proximal to stylet, anal sternite slightly rounded with numerous long bristles on border. Stylet about 3 times longer than wide, with a long terminal and three quite long subterminal or lateral bristles. Three long antepygidial bristles on each side, median one longest. Head of spermatheca barrel shaped, 1½ times longer than wide, tail curved dorsally, tip well-sclerified.

Allotype female from *Sciurus griseus* Riverside County, California, September 1936.

Male

The distinguishing characters of the male are: a row of long bristles on the broadly rounded ventral margin of tergite VIII, movable process of the clasper rectangular and armed with a long sharp spine at the posterior dorsal apex, a short stout spiniform in the middle and another at the posterior ventral angle.

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[1] Contribution from the Division of Infectious Diseases, National Institute of Health, Rocky Mountain Laboratory, Hamilton, Montana.

[2] The writer is indebted to Gretchen Gayhart Jellison for inking and lettering of the camera lucida drawings.

Specimens examined: One male from *Sciurus griseus,* Seven Oaks, San Bernardino County, California, May 1936, collected by Glen M. Kohls. One male and 1 female (allotype) from *Sciurus griseus,* Riverside County, California, September 1936, received from Dr. C. R. Eskey of the San Francisco Plague Laboratory of the U. S. Public Health Service.

This is a large, darkly pigmented species and its normal host appears to be the western grey squirrel, *Sciurus griseus.*

Opisodasys robustus (Jordan) 1925

Jordan described the female from specimens collected at White River, Navajo County, Arizona (host unknown), and at Riti de los Frijoles (Sandoval County), New Mexico, from *Sciurus aberti.* No further collections have been recorded and the male is undescribed.

The female is readily recognized by the deep incision and long, broad, obliquely truncated lobe of sternite VII. The head of the spermatheca is considerably narrowed anteriorly.

Male

Head: Frontal tubercle small, antennal groove reaching but not continuous across top of head. Bordered dorsally with fine setae. Bristles on segment 2 of antennae about ½ length of club. Eye well pigmented, gena broad. Genal row of 3 bristles, median one smallest. Two short bristles anterior to eye. Occiput with 1 medium bristle near middle of antennal groove, and 3 bristles in posterior row, ventral one longest. Rostrum equaling trochanter.

Thorax: 19 or 20 spines in pronotal comb. Two or 3 erect bristles on each notum. First pair plantar bristles displaced ventrally on all tarsi.

Abdomen: Apical spines on each side of tergites I to VI, 3, 3, 3, 3, 1 and 1 respectively. On tergites II to IV anterior row of small bristles reduced to 3, 1 and 1 respectively. Tergites II to VII with medium row of 5 or 6 long bristles on each side alternating with very fine setae.

Modified segments: Tergite VIII broadly rounded, with 12 long bristles on ventral margin, 2 long and 1 medium bristles on posterior margin and 6 long and 8 small bristles on dorsal margin. Three antepygidial bristles on each side. Middle one longest, inner one very short. Sternite VIII small, slender with fine bristles along distal half. Basal membranous appendage or plume long and slender. Proximal lobe of sternite IX with 2 pale spiniforms. Distal lobe 3 times longer than broad, acute ventrally, squarely truncate dorsally, heavily armed with numerous pale spiniforms along posterior border. Anal sternite long narrow, curved, densely bristled. Immovable process of clasper rounded apically with 4 or 5 small bristles at apex. Movable process longer than immovable process, roughly semicircular, armed with a small pale spiniform at apex and 3 dark spiniforms well-separated on posterior margin. Upper spiniform long and sharp with thick base, middle spiniform shortest, ventral spiniform of medium length.

Allotype male from fox squirrel, *Sciurus niger,* White House Canyon, Santa Rita Mountains, Santa Cruz County, Arizona, May 1933 collected by Glen M. Kohls.

The normal hosts are possibly either fox squirrels, *Sciurus niger,* or tuft-eared squirrels, *S. aberti,* or both.

Specimens examined: One male (allotype) and one female from fox squirrel, *Sciurus niger,* Santa Rita Mountains, Santa Cruz County, Arizona, May 1933, collected by Glen M. Kohls. One female from *Marmota flaviventris* (accidental host), Pagosa Springs, Archuleta County, Colorado, September 1931, collected by R. A. Cooley.

Opisodasys vesperalis (Jordan) 1929
Genotype

The male and female were described by Jordan (1929) and figures given of the male clasper and sternite VII of the female. His type series had been collected from *Glaucomys sabrinus* (=*Sciuropterus alpinus*) at Okanagan Landing, British Columbia. A series of this species is available for study which was recorded under the name of *C. acasti* by Dunn and Parker (1923). They found 51 specimens on 7 flying squirrels, *Glaucomys sabrinus* in Ravalli County, Montana.

Although the females of *vesperalis* are very similar to *pseudarctomys* the shape of sternite VII is quite constant and is a reliable character for the identification of *vesperalis*. The males are readily distinguished by having a long and short spiniform on the posterior ventral lobe and a larger saber-shaped spiniform at the posterior dorsal angle of the moveable process of the clasper. Males of *vesperalis* and *pseudarctomys* differ from others of the genus by having the acetabular bristles placed on a distinct process and by the presence of small paired lateral plates or flaps at the apex of sternite VIII.

Specimens examined: Three males and 10 females from *Glaucomys sabrinus* Ravalli County, Montana, 1918 and 1919 collected by L. H. Dunn and R. R. Parker. Three females from *Glaucomys sabrinus* Ravalli County, Montana, 1934, 1935, collected by the writer. Twenty males and 20 females from *Glaucomys sabrinus* Washington County, Oregon, February 1938, collected by C. A. Hubbard.

Opisodasys pseudarctomys (Baker) 1904

The types described and figured by Baker (1904) were collected from *Marmota monax* (=*Arctomys monax*) Herkimer County, New York, but the true hosts are flying squirrels, *Glaucomys*. Rothschild (1905) described and figured as *C. acasti* a single female collected from *Glaucomys sabrinus* (=*Sciuropterus alpinus*) at Quesnel, British Columbia. This was reduced to a subspecies of *pseudarctomys* by Baker (1905). Jordan (1929) compared the types of the two species and reduced *acasti* to synonomy. He also questioned the locality record of *acasti* as British Columbia, but added a record from the adjacent province of Alberta. Specimens from *Glaucomys sabrinus,* Ravalli County, Montana, determined as *acasti* by Dunn and Parker (1923) are of the closely related species *vesperalis*.

Both sexes have been well-described and figured by Baker and the female described in detail by Rothschild. The long slender head of the spermatheca distinguishes females of *pseudarctomys* and *vesperalis* from others of the genus. *Pseudarctomys* is readily separated by the shape of sternite VII which lacks the broad, rounded posterior dorsal lobe. The

genital characters of the male are very distinctive especially the long acetabular process, paired apical plates on sternite VIII and long slender movable process of the clasper. The latter is concave on the posterior border, the upper angle is armed with a heavy curved spiniform and the ventral portion is expanded into a rounded lobe and armed with 2 equal or subequal spiniforms of moderate length.

Specimens examined: One male from *Sciurus* sp. Alberta, Canada, 1912, collected by A. D. Gregson, received through the courtesy of Dr. Karl Jordan. One male and 1 female from *Glaucomys sabrinus,* Cloquet, Carlton County, Minnesota, Sept., 1933.

Opisodasys keeni (Baker) 1896

Baker (1896) described both the male and female from specimens collected on *Peromyscus keeni* (=*Sitomys keeni*) at Masset, Queen Charlotte Island and later (1904) supplemented the description with figures. It is recorded from *Peromyscus maniculatus* at Aspen Grove, British Columbia, by Wagner (1936).

Peromyscus appears to be the normal host of this species though it may be expected to occur on other mice.

This is the smallest species of the genus and may be readily distinguished from others by having two rows of bristles on the gena and by modified abdominal segments, which are figured.

Typical females show a semicircular incision in the posterior margin of sternite VII, with an acute upper lobe. This is reduced or absent in a few specimens. The ventral apical projection on tergite VIII separates the male from others of the genus.

Specimens examined are all from *Peromyscus* and were collected in the following localities:

Two males and 6 females from Alpine Co., California, June, 1936. One female from Alameda County, Calif., June, 1936. Two males from Coos County, Oregon, July, 1935. Three males, 4 females from Douglas County, Oregon, August, September, October, 1935. One female from Josephine County, Oregon, August, 1935. Fifteen specimens from *Peromyscus maniculatus,* Ravalli Co., Montana, May 12, 1938.

The similarity in the movable process of the claspers of males of *O. keeni* and *O. cnoplus* is very striking, although the latter is a much larger species.

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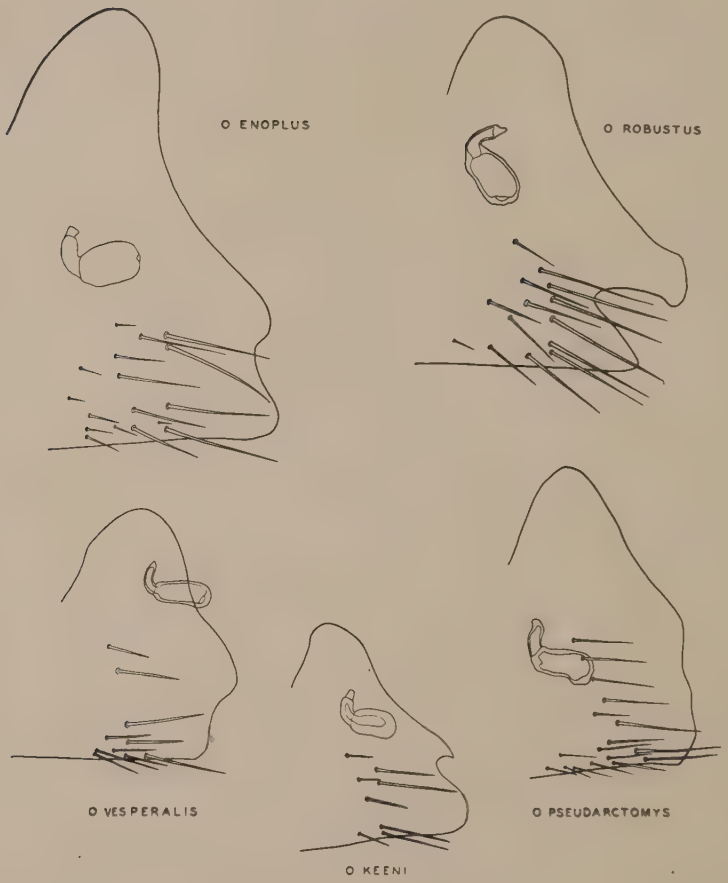
EXPLANATION OF PLATES

PLATE I. Receptaculi and sternites VII of females.
PLATE II. Sternites VIII of males.
PLATE III. Claspers of males.

Specimens figured

O. enoplus. Male and female, from *Sciurus griseus,* Riverside County, California.
O. robustus. Male and female, from *Sciurus niger,* White House Canyon, Santa Rita Mountains, Santa Cruz County, Arizona.
O. vesperalis. Male and female from *Glaucomys sabrinus,* Ravalli County, Montana.
O. pseudarctomys. Male from *Sciurus* sp. Alberta, Canada. Female from *Glaucomys sabrinus,* Carlton County, Minnesota.
O. keeni. Male and female from *Peromyscus* sp. Douglas County, Oregon. (Specimens of this species were compared with types at the U. S. Nat. Mus. by E. A. Chapin.)



RECEPTACULI AND STERNITES VII OF FEMALES

PLATE I

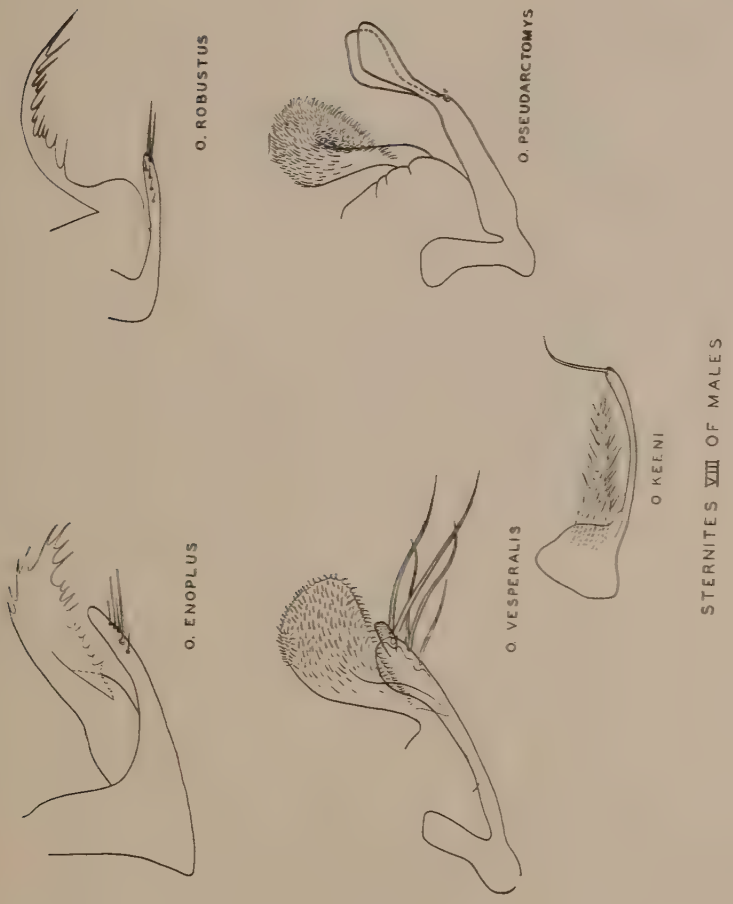
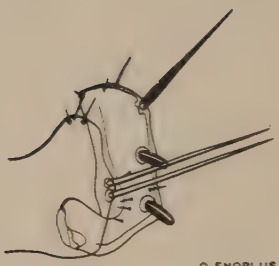
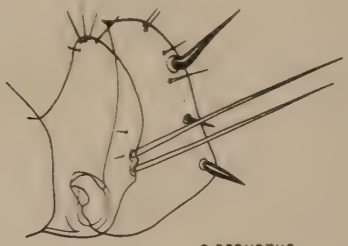

O. ROBUSTUS
O. PSEUDARCTOMYS
O. ENOPLUS
O. VESPERALIS
O. KEENI
STERNITES VIII OF MALES

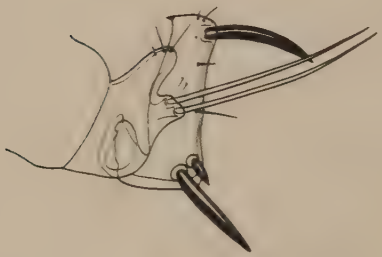
PLATE II



O. ENOPLUS



O. ROBUSTUS



O. VESPERALIS

O. PSEUDARCTOMYS

O KEENI

CLASPERS OF MALES

PLATE III

TAMERLANIA MELOSPIZAE N. SP. (TREMATODA: EUCOTYLIDAE) WITH NOTES ON THE GENUS[1]

LAWRENCE R. PENNER[2]

A new trematode of the genus *Tamerlania*, family EUCOTYLIDAE, is here described. This is the first member of the genus to be reported from North America and the fifth described species.

Genus *Tamerlania* Skrjabin 1924

Skrjabin established the family EUCOTYLIDAE in 1924 to include *Eucotyle* Cohn (1904), *Tanaisia* Skrjabin (1924), and *Tamerlania* Skrjabin (1924). Nezlobinski (1926) added the genera *Ohridia* and *Lepidopteria*. The genus *Tamerlania* contains four species, *T. zarudnyi* Skrjabin (1924), *T. meruli* Nezlobinski (1926), *T. bragai* Dos Santos (1934), and *T. japonica* Yamaguti (1935). Skrjabin lists *Passer montanus* (Linn.), the European tree sparrow, as host for *T. zarudnyi*. It was collected in 1921 by the Fifth Helminthological Expedition at Taschkent, Turkestan. Kalantarian (1924) and Khitrowo-Kalantarian (1924) list *Fringilla coelebs* L., the chaffinch, and a woodpecker, *Dendrocopus syriacus* Hempr., from Erivan, Armenia. Khitrowo-Kalantarian also reports the finding of this parasite by Issaitschikow in the rook, *Corvus frugilegus* Brehm, from Krim. It is also recorded by Skrjabin and Massino (1925) from *Monedula turrium* Brehm taken near Moscow on May 21, 1921. In this reference these authors refer to the previous host recorded by Skrjabin (1924) as *Passer domesticus* instead of *Passer montanus*. *T. meruli* is from *Turdus merula* taken at Juzna, Serbia, in the Macedonian region. *T. bragai* is described as a parasite of *Columba livia* and *Gallus domesticus* in and near Rio de Janeiro, Brazil. *T. japonica* was taken in the Japanese hawfinch, *Coccothraustes coccothraustes japonicus* Temm. and Schleg. from Mie Prefecture and in a bunting, *Emberiza variabilis* Temm. from Koti Prefecture, Japan.

Skrjabin (1924) indicated the salient features of the genus *Tamerlania* to be EUCOTYLIDAE with no anterior muscular prominence (Muskelwulst) separating the head region from the remainder of the body; esophagus absent; symmetrically situated testes with entire margins, lying just inside the intestinal crura at the junction of the anterior with the middle third of the body; ovary with entire margins; vitellaria in the middle third of the body; kidney parasites of birds. The species herein described falls within the genus as defined by Skrjabin (1924), except for

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[1] Contribution from the Department of Zoology, University of Minnesota.

[2] The writer is greatly indebted to Dr. Wm. A. Riley for his valuable criticism during the course of this investigation.

the presence of a short esophagus. The diagnosis of the genus has since been either emended or amplified by three different authors, Khitrowo-Kalantarian (1924), Dos Santos (1934), and Yamaguti (1935). Khitrowo-Kalantarian noted that the intestinal crura were united in the posterior region of the body and added this character to the generic diagnosis. Dos Santos amplified the genus to include forms with vitellaria in both the anterior and middle regions of the body. Yamaguti, apparently not cognizant of the two previous changes emended the diagnosis to include the posterior cecal connection and the presence of a short esophagus. In the species described in this paper a posterior crural union cannot be seen. A connection could not be determined when the parasite was examined by polarized light. Cheatum (1938) used this method in locating the place of union in the posterior part of the body of *Tanaisia pelidnae* Cheatum. Dos Santos does not discuss a posterior crural connection in describing *T. bragai* and in a figure shows no connection although the crura are seen bending towards each other. It is very probable that they do unite and are obscured by eggs in the convoluted uterus which invades that region. Apparently the genus *Tamerlania* is closely related to *Tanaisia*. The latter genus has an esophageal bulb whereas is *Tamerlania* there is no expansion of the very short esophagus when present. The testes in *Tamerlania* are smoothly emarginate and at about the same horizontal level while in *Tanaisia* they are lobed and either tandem or oblique. The only difference noted between *Tamerlania* and *Ohridia* is in the position of the testes and it is very possible that the lone species, *O. panuri* Nezlobinski (1926), should be included in *Tamerlania*. However, until these relationships can be further studied, *Ohridia* stands as a valid genus.

The following generic diagnosis will suffice for the described species of the genus.

Tamerlania Skrjabin, 1924, emended diagnosis

Eucotylidae with no anterior prominence separating cephalic region from remainder of body; esophagus short or absent; testes smoothly emarginate, lying at approximately same horizontal level just inside intestinal crura at junction of anterior with middle third of body; ovary anterior to testes and has entire margins; vitellaria lie outside of crura and usually in middle third of body; intestinal crura may or may not unite in posterior region of body; kidney parasites of birds.

Tamerlania melospizae n. sp.

(Fig. 1)

Specific diagnosis: Tamerlania. Body flattened, elliptical, with minute spines or scales; 3.7 mm long × 0.55 mm wide at level of testes; oral sucker sub-terminal, broader than long, 0.23 mm × 0.26 mm; pharynx broader than long, 0.08 mm × 0.09 mm; short esophagus present; intestinal crura approach caudal end of body with union, if any, obscured by eggs in the uterus; testes in middle third of body situated horizontally just inside the crura; right testis larger and slightly anterior to left testis, not lobed; right testis extreme length, 0.14 mm, extreme width, 0.15 mm; left testis, extreme length, 0.11 mm, extreme width, 0.12 mm; ovary with margins entire, anterior to right testis and near right side of body, broader than long, extreme length, 0.14 mm, extreme width, 0.23 mm; vesicula seminalis almost

median and adjacent to anterior border of ovary, oval, broader than long, 0.05 mm by 0.09 mm; receptaculum seminis oval, adjacent to right posterior border of ovary, 0.08 mm long × 0.09 mm, lateral margin partly covers right intestine; vitellaria lateral, extra-cecal, in middle third of body, glands of equal length, 1.3 mm, beginning at a level just anterior to the level of the testes; vitelline reservoir median, obscured by uterus just anterior to right testis; uterus extensively coiled filling body posterior to testes, crossing to left side of body between the testes, con-

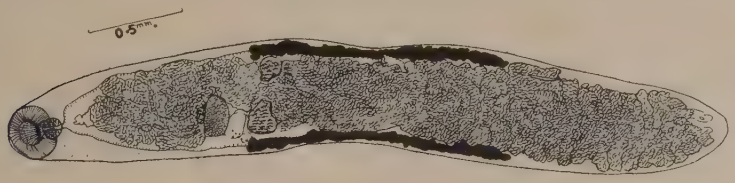

FIG. 1. *Tamerlania melospizae* n. sp., ventral view.

tinuing to esophageal region in compact coils which fill the body between ovary and bifurcation of intestinal crura; genital pore obscured, apparently just anterior to ovary behind intestinal bifurcation; eggs dark brown, operculate, symmetrically oval, 30 × 21 microns.

Host: Melospiza lincolni lincolni (Audubon).
Habitat: Urinary tract.
Locality: Minneapolis, Minnesota.
Type Specimen: U. S. Nat. Mus. Helm. Coll. No. 9288.

One specimen was found in the ureter when bits of the urinary duct and kidney of *Melospiza lincolni lincolni* (Audubon) were pressed between two glass slides and examined with a binocular dissecting microscope. The bird host was picked up dead by Max Partch, a student at the University of Minnesota, on May 1, 1938, and brought to W. J. Breckenridge of the Natural History Museum.

As only one specimen was taken no sections were made. The extensive coils of the uterus obscured various structures, particularly the genital pore and the intestinal crura. A place of union in the posterior part of the body could not be discerned with certainty.

KEY TO SPECIES OF *Tamerlania* SKRJABIN, 1924

1. Vitellaria in middle third of body; oral sucker 0.15–0.30 mm 2
   Vitellaria in anterior and middle thirds of body; oral sucker 0.40 × 0.50 mm
   T. bragai Dos Santos (1934)
2. Esophagus present; testes broader than long 3
   Esophagus absent; testes spherical 4
3. Vitellaria of equal length, 1.7 mm; ovary slightly oval; body length, 3.2–5.3 mm
   T. zarudnyi Skrjabin (1924)
   Vitellaria of unequal length, left 0.6 mm. right 0.9 mm; ovary triangular in shape; body length, 2.5 mm ..... *T. meruli* Nezlobinsky (1926)
4. Ovary considerably broader than long; eggs 30 × 21 microns
   T. melospizae n. sp.
   Ovary spherical; eggs at least 42 × 24 microns ... *T. japonica* Yamaguti (1935)

Attention should be called to the fact that the figure of *Tamerlania meruli* Nezlobinsky was interchanged with *Lepidopteria plegadis* Nezlobinsky in the original publication.

DISCUSSION

The host relationships of the members of this genus are interesting. *T. zarudnyi* has been found in the urinary passages of the chaffinch, rook, European tree sparrow, and others. *T. meruli* is from a blackbird of Europe. *T. japonica* utilizes the Japanese hawfinch, a bunting, and probably other birds as its hosts. *T. bragai* occurs in 15.7 per cent of the pigeons of Rio de Janeiro and causes dilation and hyperplasia of the collecting tubules. The ducts become enormously enlarged and filled with amorphous and crystallized detritus. Dos Santos (1934) found that the parasite rarely invaded the cortical substance of the kidney. He also found the kidneys of two hens infected. To date the species herein described has been taken from but one host, the Lincoln sparrow, a migrant through Minnesota. It is interesting to note that one of the hosts of *T. zarudnyi,* the European tree sparrow, was liberated in this country in Missouri where it became acclimatized. Barrows (1889) records twenty specimens from Germany liberated at LaFayette Park, St. Louis, Mo., in 1870. Its very close relative, the English sparrow, also an imported species, had been liberated in St. Louis the year before. In discussing the range of the latter species Hellmayr (1938) records it from Rio de Janeiro, Brazil. It is quite probable that more careful examinations of the urinary passages of English sparrows and domestic fowl in this country will reveal more information on these potentially dangerous fowl parasites.

SUMMARY

A kidney trematode, *Tamerlania melospizae* n. sp., is described from the Lincoln sparrow, *Melospiza lincolni lincolni* (Aud.), with a history of the genus and a discussion of its occurrence. A key to the known species is also appended.

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A STUDY OF THE RELATION OF VITAMIN A TO LOUSE RESISTANCE IN RATS*

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In a study (3) of the control of ectoparasites of laboratory rats it was noted that the animals examined varied widely in the number of rat lice, *Polyplax spinulosa* Burm. which they bore. It was learned that many of the most heavily infested rats had been maintained on a vitamin A deficient diet. This information, combined with the common observation that domestic animals in poor flesh and poorly fed, are frequently heavily infested with lice, particularly in the early spring, suggested a study of the relation of diet to resistance to these ectoparasites.

Our study deals with the relation of vitamin A to the resistance of laboratory rats to infestation by the rat louse. Data are presented which tend to prove that vitamin A is effective in conditioning rats against infestation by the rat louse. It is also indicated that rats suffering from vitamin A-avitaminosis are susceptible to infestation.

MATERIALS AND METHODS

First Period of Study

During October 1936, 72 young rats of about 50 grams each were placed in two large colony cages with one rat in each cell. They were divided into 3 groups according to the treatment they were to receive. All were given a basal synthetic ration (4) low in vitamin A. Twelve rats were given supplemented feedings of 20 micrograms of vitamin A daily from the start. They were used as controls and were placed in the lowest tier of cells in each colony cage in order to isolate the test rats from all sources of vitamin A, particularly coprophagy. Vitamin A was administered by means of a medicine dropper, as carotene dissolved in Wesson oil. Stock supplies adjusted to contain 1½, 3, 10, or 20 micrograms per drop were used. The 60 test rats were continued only on the deficient diet until they showed definite symptoms of vitamin A deficiency. These symptoms were those described by Gudjonsson (2) and included constant weight, flecks of foreign materials in the eyes, soiled tails, and loss of hair from the eyelids.

When these symptoms were observed, all of the rats were infested

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with about 20 rat lice per animal. Ten rats were then given 20 micrograms of vitamin A per day while the other 50 were continued as before. Depleted rats which became seriously ill were given 1½ or 3 micrograms of vitamin A daily, depending on the severity of the illness, until they recovered to the general condition of the other depleted rats or died. Three additional attempts were made to colonize lice upon the check animals. The rats were examined daily and notes were made on the condition of each rat and the relative number of lice found.

In recording infestation, the rats were carefully examined by pressing back the hair, exposing the skin and noting the relative number of lice. This was recorded as either none, few, many, or severe. Few was used to indicate a recognizable number and varied from 1 to 20. Many indicated greater numbers than could be kept separated for counting. Severe indicated great numbers. Lice to the numbers of from 6,000 to 17,000 were brushed from dead rats which had been recorded as severe.

Second Period

Seventy-two young female rats, about 50 grams in weight, were placed in the 2 large colony cages on Oct. 18, 1937. The rats in the lowest 6 cells in each cage were given 20 micrograms of vitamin A daily and used as checks, as before, while all the others were used as test animals. All were given the basal synthetic diet low in vitamin A. For the first 30 days, the test rats were given 1½ micrograms of vitamin A daily. At the end of about 50 days, vaginal smears (1) were made daily from each rat. The vaginal smear method for detecting vitamin A depletion in rats was very useful and was depended upon to indicate when the rats were depleted of vitamin A or when they had obtained a sufficient quantity to resume their normal cycle. By this time, all the test animals had reached a constant weight. They were still following their normal estrual cycle. When the test animals persisted in the estrus stage for about one week, they were considered as depleted of vitamin A and were infested with about 20 rat lice per rat. This stage was reached at the end of about 60 days from the day they were received. Each rat was given 20 additional lice 30 days later. Each day until the experiment was ended or until the animals died, vaginal smears were made and notes taken on the general appearance of the animals. Data on the weight of the rats were taken on days alternating with the infestation notes. Lice were placed on the check rats, four times in all. Ten of the 41 rats which survived to nearly the end of the experiment developed large infestations and were given 60 micrograms or more of vitamin A daily after the infestation became heavy.

If a depleted rat became ill or lost weight rapidly for a few days, vitamin A was administered until the animal recovered or died. With the exceptions noted, there was no case in which a sufficient amount of vitamin A was given to cause a return to the normal estrual cycle. During both periods, it was necessary to terminate the experiments about June 1.

EXPERIMENTAL RESULTS

First Period

The rats which were given the abundant supply of vitamin A and served as controls grew quite normally and were free from lice throughout the experiment except for a day or so immediately after each time they were infested.

At the end of the experiment, 35 rats remained alive of the 50 animals which had been placed on the low vitamin A ration. All of these were emaciated, had harsh, wiry hair, soiled tails, and accumulations of matter about the eyes. Many were quite blind. Eleven were severely infested, 10 had many lice, and 14 had few lice.

Of the 10 rats which had been given an abundance of vitamin A as soon as they were depleted of vitamin A and infested with lice, 2 died, 3 did not recover their normal condition but lost weight. They also had eye disorders, bristly fur, dirty noses, and carried a few lice. The others were quite normal in appearance and had gained weight. The 8 surviving rats, including the 3 mentioned above which did not recover, bore either few or no lice.

All of the surviving test animals showed obvious evidence of the deficiency. Some suffered hemorrhages from the nose, some had diarrhea, others had less severe symptoms. Criteria, by which depletion could be recognized, were abundant, but it sometimes appeared that the rats showed symptoms of disease which appeared secondary to or associated with the vitamin A depletion. Many of the test animals died. In most cases, death was due to pneumonia to which they were very susceptible. They sometimes recovered to contract it the second time. In many cases, a small supplemental feeding of vitamin A was sufficient to restore the rats even though they had pneumonia.

Second Period

The history of the check rats was essentially the same as that in the previous experiment. These rats, with the abundant supply of vitamin A, grew quite normally and successfully repelled all lice which were placed upon them. It is probable that there was some migration of lice about the cages, for the check animals, although usually quite free from lice, sometimes carried one or two.

About 30 days after the vaginal smears showed definite lack of vitamin A and the rats were given the first infestation, most of the rats showed obvious signs of disease. Many had had diarrhea, some had bled from the nose. Nearly all had shown less severe symptoms such as reddening of the lids, accumulations of matter about the eyes, filth-covered tails, and epilation.

By this time many of the test rats had developed significant infesta-

tions of lice. Some, however, had not accepted an infestation. They all accepted the second infestation of lice which was placed upon them at this time, thereby indicating they had become susceptible. Thirty days later, all of the surviving test rats bore significant numbers of lice.

Ten of the test rats became heavily infested about 90 days after the vaginal smears showed that the rats had reached a constant estrual condition. Here again, there was no obvious symptom of susceptibility. It was found that in 7 of the 10 cases, 60 micrograms of vitamin A daily was sufficient to dispel the infestation after about 30 days. The rats returned to their normal estrual cycle in the meantime. One of the 10 heavily infested rats developed the infestation so late in the season that the experiments had to be discontinued before the effects of the large doses of vitamin A appeared. The other two died before the infestations were obviously reduced. Four of this group of 10 died after they had reduced the heavy infestation to few or none. The cause of death was not determined.

DISCUSSION AND CONCLUSION

This study has indicated that in this case vitamin A was the chief factor controlling the susceptibility of the rats to the rat louse. After they had been depleted of their vitamin A stores, all of the rats which were held on the vitamin A deficient ration accepted and maintained infestation of lice in varying degrees. It is also clearly indicated that all of the rats did not become completely susceptible to the lice as soon as their stores of vitamin A were depleted. Many of them maintained small but persistent infestation for some time after they were infested before their resistance was completely destroyed and heavy infestations developed. There appeared to be two distinct degrees of susceptibility. Soon after the rats exhibited definite vitamin A-avitamionsis, they accepted and maintained some lice. They appeared at this stage to exercise some control over the lice. In the second stage, the rats appeared to lose all control over the lice and became heavily infested.

After varying periods of vitamin A starvation, many of the rats exhibited symptoms of disease resulting from or associated with vitamin A-avitaminosis. These secondary diseases may have been closely associated with the susceptibility of the rats to lice. There was no obvious symptoms of susceptibility beyond the general symptoms of vitamin A-avitaminosis. Rats in all degrees of health from large, vigorous, ones to thin, weak-looking ones, accepted varying degrees of infestation. Many of the rats which became severely infested had rough dry hair. This was thought to be associated with the susceptibility to lice. Rats which received an abundance of vitamin A and were resistant to lice had glistening, fully furred coats which felt silky or oily. Heavy infestations did not occur on all of the rats which developed harsh, dry, thin coats,

however. Thin, light rats were not more susceptible than heavy, fat rats. The function of the various physiological processes of the rats in making them resistant or susceptible to infestation were not studied. Lack of appetite or sluggishness of movement or other disorders resulting from vitamin A-avitaminosis may have exerted considerable influence on the susceptibility of the rats.

Lice did not colonize upon the check rats which had received large quantities of vitamin A from the beginning of the study despite several attempts to start infestations. From this it may be concluded that vitamin A was the main factor which regulated the resistance or susceptibility of the rats to the lice. Of the 10 rats in the first period which were given adequate amounts of vitamin A after they had been depleted and infested, all 8 surviving rats were successful in holding the infestation to at least few. The lice completely disappeared from 6 of these. In the second period, ten of the 41 test rats surviving 90 days or more after they were infested became completely susceptible and heavy infestations developed. When the infestations became heavy and vitamin A was supplied, the infestations on 7 of the 10 were reduced to few or none. In the meantime, they returned to their normal estrual cycle. Lice numbers were not reduced on the test rats unless they were given an adequate quantity of vitamin A. The small amounts of vitamin A which were given to correct severe disease conditions did not reduce the number of lice or return the normal cycle of the rats.

SUMMARY

1. Young rats deprived of vitamin A soon developed symptoms of disease which were recognized as associated with vitamin A deficiency.

2. When these disease symptoms were observed and the rats were artificially infested with rat lice, all of the surviving rats on the vitamin A deficient rations accepted and maintained some degree of infestation.

3. Twenty-one of 76 surviving rats on the vitamin A deficient diet lost control of the infestations after varying periods of time and became heavily infested.

4. Seven of the 10 rats which became heavily infested in the second period were able to dispel the infestation when given an abundance of vitamin A.

5. Few lice were found on the survivors of the 10 rats in the first experiment which were given the abundance of vitamin A after they had been depleted and infested. The numbers were so small and infrequent that they might have been accounted for by migration from surrounding rats.

6. Rats which received an abundance of vitamin A throughout the experiments were uniformly free from lice despite several attempts to colonize lice upon them.

7. It is indicated that lack of vitamin A caused the rats to become susceptible to louse infestation while an abundance of vitamin A made them resistant.

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RICTULARIA ONYCHOMIS N. SP. (NEMATODA: THELAZIIDAE) FROM THE GRASSHOPPER MOUSE, *ONYCHOMYS LEUCOGASTER* (WEID)*

ASHTON C. CUCKLER

On October 15, 1936, three mature, female nematodes of the genus *Rictularia* Froelich, 1802, of the family THELAZIIDAE Railliet, 1916, were obtained from a grasshopper mouse, *Onychomys leucogaster* (Weid), collected near Valentine, Cherry County, Nebraska, by Mr. Edson Fichter, University of Nebraska. These parasites were deeply embedded in the wall of the intestine. The specimens were fixed in hot 70 per cent alcohol and cleared and mounted in glycerine for study. Comparison with the known forms indicates these specimens constitute an undescribed species for which the name *Rictularia onychomis* is proposed. The following description is based entirely upon females, since no males were collected.

Rictularia onychomis n. sp.

(Figs. 1–6)

Specific diagnosis: Rictularia. Females only available.

Female: Length 24–42 mm; maximum diameter 0.39–0.46 mm in posterior third of body. Cuticle variable in thickness. Two rows of ventral cuticular processes, beginning 0.10–0.11 mm from anterior end and extending to anterior of last 8th or 9th of body length. The anterior processes are blade-like and with continuous bases. These are called combs, of which there are 26–27 pairs. The length of the combs gradually increases from the anterior one to the 20th, and then decreases from this comb to the last (Fig. 2). Posteriorly the processes are slender and spine-like, and without continuous bases. These processes are termed spines, of which there are 30–33 pairs. There is a total of 56–60 pairs of cuticular processes. Mouth terminal, hexagonal in shape, apparently bounded by 6 rudimentary lips. Ventrally the indentations between the lips are conspicuous, but dorsally they are not apparent (Fig. 4). Anterior portion of stoma surrounded by a ring of 26 denticles, variable in size and shape. Stoma 0.07–0.08 mm in depth by 0.09–0.10 mm in diameter. Walls of stoma well sclerotized. Three tooth-like projections extend from base of stoma towards mouth. Amphids terminate near bases of lips. Two circles of cephalic papillae; external circle apparently of 4 papillae situated some distance from edge of lips; internal circle apparently of 4 (?) papillae near edge of lips (Fig. 4). Nerve ring 0.33–0.37 mm from anterior end. Excretory pore not observed. Esophagus 4.5–5.0 mm × 0.08–0.13 mm, apparently same structure throughout. Esophago-intestinal valve distinct. Intestine rectilinear. Tail tapering, 0.48 mm long (Fig. 5). Vulva (Fig. 1) 3.9–4.7 mm from anterior end, between 6th and 7th pairs of spines, or the 32nd–33rd pairs of cuticular projections. Uteri filled with thick-shelled embryonated eggs, which average 45 × 33 microns in size.

Host: Onychomys leucogaster (Weid), the grasshopper mouse.

Location: Intestine.

Type locality: Valentine, Cherry County, Nebraska, U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9289.

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*Contribution from the Department of Zoology, University of Minnesota. The writer expresses his appreciation to Dr. W. A. Riley for helpful criticisms.

This species can be distinguished from the other species of *Rictularia* on the basis of its size, the total number of cuticular processes, the number of prevulvar cuticular processes, the position of the vulva anterior to the posterior end of the esophagus, and the terminal mouth opening. This species resembles *R. elvirae* Parona, 1889, in the number of cuticular processes, but it is without cervical alae, and the diameter of the body and the size of the eggs is much less. *R. onychomis* also resembles *R. taterilli* Baylis, 1928, in the total number of cuticular processes, but the number of prevulvar combs is considerably less. Apparently 23 species of *Rictularia* have been reported from various parts of the world as parasites of rodents, carnivores, bats, and a lizard. Three species have previously been reported from the United States. *R. onychomis* is distinctly different from these latter species in size, number of cuticular processes, and position of vulva.

The species of *Rictularia* may be differentiated with the following key, which is based entirely upon the females, since no males are known for more than one-half of the species. All species encountered in the literature are included in the key. One species, *R. disparilis* Irwin-Smith, 1922, from *Hinulia* sp., a lizard, probably does not belong to this genus, since there is only a single ventral cuticular ridge with a double row of spine-like processes. Other species are very inadequately described, and their status is also in doubt. However, they are all included in the key, since it has not been possible to pass on their validity.

KEY TO THE SPECIES OF *Rictularia* BASED UPON FEMALES

1. Adult female usually much less than 15 mm long 2
   Adult female usually much more than 15 mm long 7
2. Insufficiently described to characterize further with key
   R. boveri R. Blanchard, 1886
   Sufficiently described to characterize further with key 3
3. With a single ventral cuticular ridge with two rows of spines
   R. disparilis Irwin-Smith, 1922
   With two ventral cuticular ridges each with a row of spines 4
4. With more than 100 combs and spines 5
   With less than 100 combs and spines 6
5. Vulva posterior to posterior end of esophagus; with 46–52 prevulvar combs
   R. cahirensis Jägerskiöld, 1904
   Vulva anterior to posterior end of esophagus; with 55 prevulvar combs
   R. splendida Hall, 1913
6. Vulva at level of posterior end of esophagus ..... *R. coloradensis* Hall, 1916
   Vulva considerably posterior to posterior end of esophagus
   R. elegans Travassos, 1928
7. With more than 100 combs and spines ..... *R. affinis* Jägerskiöld, 1904
   With less than 100 combs and spines 8
8. Position of vulva definitely known 9
   Position of vulva not definitely known 22
9. Vulva anterior to posterior end of esophagus 10
   Vulva posterior to posterior end of esophagus 17
10. With more than 60 combs and spines 11
    With no more than 60 combs and spines 14

11. With 30–32 prevulvar combs ..... *R. macdonaldi* (Dobson, 1880)
With 41–43 prevulvar combs 12
12. With 89–93 combs and spines 13
With 77 combs and spines ..... *Rictularia* sp. (Baylis (1934))
13. Last pair of "combs" (spines?) at level of anus ..... *R. tani* Hoeppli, 1929
Last pair of "combs" (spines?) posterior to level of anus
R. whartoni Tubangui, 1931
14. Mouth terminal; with 26–27 combs ..... *R. onychomis* (this paper)
Mouth directed dorsally; with more than 26–27 combs 15
15. With less than 50 combs and spines ..... *R. proni* Seurat, 1915
With more than 50 combs and spines 16
16. With 33 prevulvar combs ..... *R. amurensis* Schulz, 1927
With 40–41 prevulvar combs ..... *R. taterilli* Baylis, 1928
17. With less than 40 prevulvar combs 18
With more than 40 prevulvar combs 19
18. With 16–18 prevulvar combs ..... *R. cristata* Froelich, 1802
With 28 prevulvar combs ..... *R. scalopis* Goodrich, 1932
19. With 75–76 combs and spines 20
With 84–97 combs and spines 21
20. With 43–44 prevulvar combs; eggs measure 38–40 × 28–30 microns
R. plagiostoma (Wedl, 1862)
With 42 prevulvar combs; eggs measure 50–60 × 32–44 microns
R. aethechini LeRoux, 1930
21. With 42 prevulvar combs ..... *R. fallax* Jägerskiöld, 1904
With 47–49 prevulvar combs ..... *R. mjöbergi* Baylis, 1928
22. With 68–75 combs and spines; 38–39 prevulvar combs .. *R. harrisi* Baylis, 1934
Apparently with less than 68–75 combs and spines 23
23. With a total of 26 combs; eggs measure 56 × 28 microns
R. elvirae Parona, 1889
With 39–40 prevulvar combs; eggs measure 53 × 40 microns
R. caucasica Schulz, 1927

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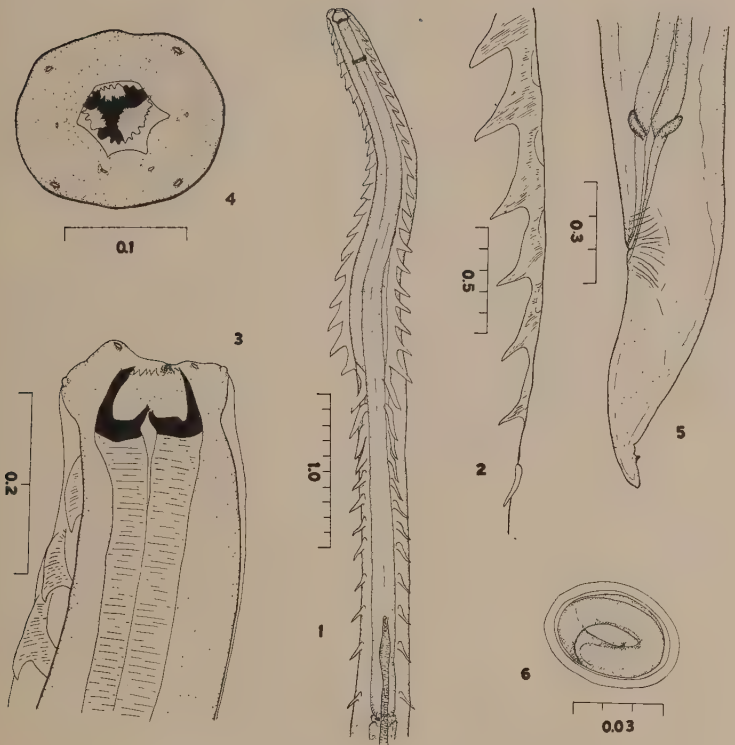
EXPLANATION OF PLATE

All figures drawn with aid of a camera lucida. Value of projected scale is in millimeters. All figures of female *Rictularia onychomis*.

FIG. 1. Anterior end, ventral view.
FIG. 2. Posterior combs and anterior spine, lateral view.
FIG. 3. Cephalic region, lateral view.
FIG. 4. Lips, *en face* view.
FIG. 5. Posterior end, lateral view.
FIG. 6. Egg from uterus.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A NEW LIVER FLUKE OF THE GENUS *OPISTHORCHIS*

F. G. Wallace and Lawrence R. Penner
Department of Zoology, University of Minnesota

An opisthorchid fluke was collected by one of us (L.P.) in the gall bladder and bile ducts of three muskrats in March and one in December, 1938. In May, 1939, metacercariae from the minnow, *Notropis deliciosus stramineus*,[1] were found by one of us (F.W.) to develop into flukes identical with those previously found in the muskrat when fed to various laboratory animals. The specific name is derived from the locality of the muskrat infections, Tonka Bay, Lake Minnetonka. The following description is based on fixed specimens from both sources, one of the muskrat parasites being designated as type.

Opisthorchis tonkae n. sp.

(Fig. 1)

Specific diagnosis: Genus *Opisthorchis*. Body flat and elongate, pointed anteriorly and rounded posteriorly. Length 3 mm to 5 mm; width 0.8 mm to 1.1 mm. Cuticula covered with spines 0.003 mm to 0.004 mm long except in a few of the larger muskrat specimens. Oral sucker 0.13 mm to 0.17 mm wide by 0.12 mm to 0.14 mm long, opening anteriorly. Acetabulum 0.13 mm to 0.16 mm wide by 0.12 mm to 0.15 mm long and located ¼ to ⅕ of body length from anterior end. Pharynx 0.08 mm to 0.09 mm wide by 0.09 mm to 0.10 mm long. No prepharynx. Esophagus 0.03 mm wide, slightly shorter than pharynx and surrounded by densely packed nuclei. Ceca of same width as esophagus for first 0.01 mm to 0.02 mm after bifurcation, then abruptly widen to 0.04 mm to 0.06 mm, and extend almost straight to posterior end of body. Genital pore median and just anterior to acetabulum. Testes tandem or very slightly obliquely placed in posterior third of body; shallowly divided into four to seven lobes, which are sometimes scarcely discernible. Posterior testis slightly larger than anterior and with more and deeper lobes. Vasa efferentia unite midway between ovary and genital pore. Seminal vesicle sinuous, begins about ¾ of the way from ovary to genital pore, and passes over acetabulum to genital pore. Ovary sub-triangular, median, and about middle of body length. Seminal receptacle posterior and dextral to ovary. Laurer's canal present. Vitellaria extracecal, in a continuous series more or less clearly definable into seven to nine groups of follicles on each side; usually extending from a point anterior to acetabulum to level of front part of posterior testis. Some variation between vitellaria of two sides of same fluke and among different flukes. Among 67 specimens, vitellaria in 35 (52 per cent) clearly pass beyond anterior margin of acetabulum on both sides; in 15 (22 per cent) vitellaria pass acetabulum only on right; in 5 (7 per cent) vitellaria pass acetabulum only on left; and in 2 (3 per cent) vitellaria do not reach beyond front margin of acetabulum on either side. Similar variations in posterior limits of vitellaria but in almost all cases they reach, at least on one side, posterior to anterior margin of second testis. Transverse uterine loops fill intercecal space from ovary to acetabulum, here and there overlapping ceca. No loops of uterus anterior to acetabulum. Excretory pore terminal. Excretory vesicle sigmoid, passing to left of posterior testis and to right of anterior testis and extending to level of seminal receptacle. Eggs 24 μ to 28 μ, average 26.3 μ long by 12 μ to 16 μ, average 15.3 μ wide.

Received for publication, June 26, 1939.

[1] The authors are indebted to Mr. Raymond L. Lindemann and Mr. Walter G. Moore for the identification of this minnow.

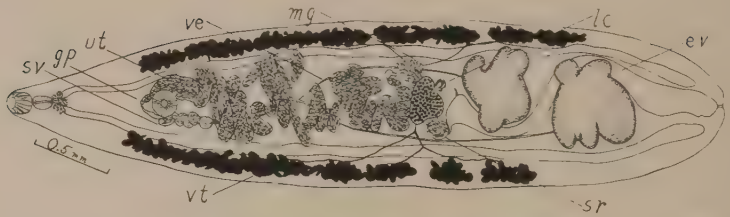

FIG. 1. *Opisthorchis tonkae,* ventral view. Abbreviations: ev—excretory vesicle, gp—genital pore, lc—Laurer's canal, mg—Mehlis' gland, sr—seminal receptacle, sv—seminal vesicle, ut—uterus, ve—vas efferens, vt—vitellaria.

Definitive hosts: Ondatra zibethica. Experimental: cat, dog, guinea pig, and white rat.

Transport host: Notropis deliciosus stramineus Cope.

Location: Bile ducts, gall bladder, and pancreas in definitive hosts. Muscles in transport host.

Localities: In muskrat: Lake Minnetonka, Minn. In fish: Lake Francis, Isanti County, Minn.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9286.

Paratypes: Dept. Zool. Univ. of Minnesota and U. S. Nat. Mus. Helm. Coll. No. 9287.

Opisthorchis tonkae agrees in all respects with the characters of the genus *Opisthorchis* R. Blanchard (1895) except in the length of the vitellaria. Erhardt (1935) cites the considerable variation in the vitelline picture of various opisthorchids and questions the validity of this character when used alone for specific or generic separation. Although there is variation in our species, the fact that the vitellaria on both sides extend beyond the anterior margin of the acetabulum in more than 50 per cent of the worms and always reach at least to the posterior margin seems to be a valid distinction from most other species of *Opisthorchis.* The vitellaria do not reach the acetabulum in any species except *O. noverca* Braun, as described by McConnell (1876), where the acetabulum is much smaller than the oral sucker.

The extension of the vitellaria beyond the acetabulum fits the diagnosis of the subfamily METORCHIINAE Lühe but the vitellaria also extend to the second testis and the uterus is not characteristic of that group in which there are numerous loops anterior to the acetabulum. In some of our larger specimens (30 days in the cat, 17 days in the guinea pig, and one muskrat infection) the uterus overlaps the ceca to a greater extent than is shown in the figure and the uterus bulges slightly anterior to the acetabulum. However Heinemann (1937) found that in *Metorchis intermedius* 13 days old there were numerous uterine loops anterior to the acetabulum. In view of this it seems improbable that the absence of pre-acetabular uterine loops in our species could be due merely to the youth of the specimens.

In the posterior extent of the vitellaria our species agrees with the genus *Amphimerus* Barker (1911) but it does not exhibit the other and

equally important character of the genus, the spaces in the vitelline chains at the level of the ovary.

While the vitelline glands do not exactly fit the present concept of any opisthorchid genus, the close agreement of our species in all other respects with the genus *Opisthorchis,* and particularly its type species, *O. tenuicollis* Rudolphi (= *O. felineus* Rivolta) leads us to include it in this genus. This will require the emendation of the generic diagnosis to include forms with vitellaria extending beyond the acetabulum anteriorly and to the second testis posteriorly.

The decision to place our species in the genus *Opisthorchis* is supported by our as yet incomplete life history data. The metacercaria enclosed in a transparent, thin-walled cyst, appears to be almost identical with that described by Vogel (1934) for *O. tenuicollis.* The cysts of species of *Metorchis* as described by Heinemann (1937) and Hsü and Khaw (1936), and that of a species of *Amphimerus* which we are now studying have distinctly thicker walls. Reviews of the family such as those of Morgan (1927) and Erhardt (1935) agree that the classification of the *Opisthorchiidae* Braun is not yet entirely satisfactory but we hope that an evaluation of the dependability of the generic characters and a natural generic allocation of this as well as other species will result from further biological studies.

No significant difference was found between specimens from natural infections in the muskrat and experimental infections in the dog, cat, guinea pig, and white rat. The size range of flukes 12 days old in the dog and white rat was 3 mm to 3.8 mm. Flukes 17 days old in the guinea pig measured 4.4 mm to 5 mm in length while those 30 days old in the cat measured 4.4 mm to 4.8 mm. In three of the muskrats the flukes were 3 mm to 3.5 mm while in one they were 4 mm to 4.5 mm.

This is the first species of *Opisthorchis* (sensu stricto) to be reported from the new world, all the hitherto described American forms in the subfamily *Opisthorchiinae* Looss having been ascribed to the genus (or subgenus of some authors) *Amphimerus.*

SUMMARY

A new species of liver fluke, *Opisthorchis tonkae,* is described from natural infections in the muskrat and experimental infections in the dog, cat, guinea pig, and white rat. It differs from all other species of *Opisthorchis* except *O. noverca* in that the vitellaria extend beyond the acetabulum anteriorly and to the second testis posteriorly. It differs from *O. noverca* in that the suckers are equal in size. The metacercaria is found in the minnow, *Notropis deliciosus stramineus.*

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RESEARCH NOTES

THE NUCLEIC ACID CYCLE IN *ENDAMOEBA BLATTAE*

Since the development of the Feulgen technique, it has been widely used as the most reliable method available for cytological work on thymonucleic acids. Although it is not truly specific, it more nearly approaches it than does any other method as yet devised. The application of Feulgen's nucleal reaction to various protozoa in recent years has brought to light the fact that although the majority of protozoan nuclei contain free thymonucleic acids certain others fail to give a positive reaction for this substance. This has lead to the supposition that thymonucleic acids are absent in the latter forms, or present in such small quantities that the color reaction on which the Feulgen test depends is not produced. This consideration has lead to the further supposition that chromatin is not necessarily composed of free thymonucleic acids in some of the Protozoa. According to the results of Morris (J. Morph. **59**: 225–63) and Sassuchin (Arch. Protistenk. **84**: 186–96) *Endamoeba blattae* is one of these.

As a result of cytological investigations extending over the past several years, it has become apparent that *E. blattae* in the interphase condition never gives a positive Feulgen reaction, but may do so in other stages. The basophilic material in the interphase nucleus, comprising a varying number of large endosomes, is never found to have free nucleic acid in its substance, either during interphase or kinetophase. The central region of the nucleus, normally acidophilic, becomes slightly basophilic during the early division stages, and thereafter shows a faint positive reaction with the Feulgen test. As the chromosomes form from this central region the coloration of its substance produced with the Feulgen test becomes increasingly dark, and is interpreted as containing an increasingly large amount of free thymonucleic acids. Ultimately the chromosomes, during their migration to the poles of the dividing nucleus, come to contain all of the thymonucleic acid demonstrable in the nucleus. During the dedifferentiation of the chromosomes the nucleic acid rapidly disappears, and when the new central region is completely formed from the chromosomal substance there is no nucleic acid demonstrable.

Such divisions occur throughout the trophic life of the amoebae, the nucleic acid disappearing during the interphase and appearing only during division. As the precystic condition begins, however, the pattern of nucleic acid distribution is wholly changed. After several typical divisions which are like those of the trophic amoebae, a nuclear transformation occurs, during which nucleic acid appears in the nucleus. It is concentrated in a small circlet of substance which is stained intensely with basophilic dyes, and which lies at the pole of the central region. This circlet ultimately contains all of the nucleic acid in the nucleus. It is interesting to notice that during the trophic stage the material which is most basophilic is distinct from the nucleic acid-containing material, but that in the precystic period the most basophilic substance is identical with the nucleic acid-containing material.

At the time of the formation of the cyst wall, the nuclei migrate to the center of the amoeba and become filled with a spongy reticulum bearing a number of small granules which are high in nucleic acid content. Following the deposition of the cyst wall there are two synchronous nuclear divisions, during which the nucleic acid content is at its peak. Following the formation of the definitive cyst nuclei the nucleic acid becomes increasingly difficult to demonstrate, and in mature cysts the nuclei are almost wholly negative to the Feulgen test.

Thus in the life cycle of *E. blattae* there are two types of cyclical variation in amount of nucleic acid present. One is associated with the division of the trophic amoebae, and the other is associated with encystment.

The failure of the basophilic material to give a positive reaction with the Feulgen test in the trophic condition, and the formation of the chromosomal material from a central portion of the nucleus which is not distinctly basophilic pre-

sent a very close similarity to the conditions observed by Chalkley (J. Morph. **60**: 13–30) during the division of *Amoeba proteus*. Sassuchin suggested that further work might reveal particular stages in the life cycle of *E. blattae* in which nucleic acid could be demonstrated, as is actually true according to the results obtained in this study. Application of the Feulgen reaction to test certain sporozoa, belonging to the genus *Eimeria*, revealed that while the nucleus was usually lacking in nucleic acid the microgamete nuclei contained enough to give a positive Feulgen reaction. Evidence is gradually accruing which suggests that in those protozoa in which nucleic acid is not invariably present some may appear during particular stages of their development, such as division or encystment or sexual reproduction. In *E. blattae*, at least, there is a definite cyclical variation in the amount of nucleic acid present which is linked with the processes of division and encystment. —Paul A. Meglitsch, *Department of Zoology, University of Illinois.*

REMOVING AIR BUBBLES FROM THE BUCCAL CAPSULES OF BURSATE WORMS

During the process of handling and clearing nematodes with well-developed buccal capsules, it is not uncommon to have air bubbles become incorporated in the capsules. These bubbles not only detract from the appearance of specimens but obscure teeth and other structures. Pressure applied to the anterior extremities of the helminths frequently results in damaging them, although the bubbles may be expelled.

A simple method of removing air bubbles and ingesta from the capsules consists in washing them with a stream of clearing solution or any other liquid medium in which the specimens are preserved. The washing may be done with a pipette, the tip of which is drawn out to a very delicate capillary tube. The worms, submerged in liquid, are held with fine forceps, a few cc of fluid are drawn into the body of the pipette, the capillary tip is inserted into the buccal capsule and a stream of fluid forced in by blowing about half the liquid out of the pipette.

Care must be taken not to introduce more air. The above process may be performed with the naked eye in the case of large strongyles, such as those from equines, and with the aid of the dissecting microscope for smaller species.—A. A. Kingscote, *Ontario Veterinary College, Guelph, Canada.*

DEMONSTRATION OF THE LIFE HISTORY OF THE NEMATODE *SYPHACIA OBVELATA* (RUDOLPHI, 1802)

Gravid female worms (recovered from the ceca of purchased white mice) were macerated in saline and fed to five white laboratory mice. Autopsy, six days later, revealed the following:

Mouse No.	*Females*	*Males*	*Total*
1	4	2	6
2	15	9	24
3	5	3	8
4	11	6	17
5	3	0	3
Totals:	38	20	58

Autopsy of three cage-mates of the experimental animals indicated the absence of natural infection with *S. obvelata.*

The life history is thus direct, without an intermediate host, as might be expected from the fact that other Oxyuroidea (e.g., *Enterobius vermicularis*) have direct life-cycles.

Of interest are the facts that more females than males were recovered, and that about one-third of the females contained eggs at this interval after infection.—H. J. Lawler, *Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University.*

SNAILS AS INTERMEDIATE HOSTS OF ACANTHOCEPHALA

Among the considerable variety of animals reported as intermediate hosts of the Acanthocephala, there seems to be no mention in the literature of snails acting in this capacity.

Recently, while examining snails for cercaria, larval acanthocephalans were obtained from two species of snails found in the Grand River near Lansing, Michigan. The snails were identified as *Campeloma decisum* and *Pleurocera acuta*. The larvae have been tentatively identified as belonging to the genus *Neoechinorhynchus*. They were found in cysts located in most cases in the foot. Visual inspection of the snails, particularly *Campeloma*, often sufficed to detect parasitized snails. The cysts could be seen as pearl-white bodies 1.0–1.5 mm in diameter along the edge of the foot. Occasionally they were located in deeper positions but always in the general vicinity of the mouth and foot. As many as fifteen larvae were found in single snails. Sometimes two were found in a single cyst. Occasionally snails were noticed in which one of the cysts had ruptured. The larva was seen hanging to the broken edge of the cyst. It could not be determined whether or not this was a normal occurrence. The most striking point in connection with the occurrence of the worms was the large number of snails found to be infected. In certain places the infection of *Campeloma* approached 90 per cent. Lack of reports concerning Acanthocephala in snails in other localities seems to discount the possibility of snails being required intermediate hosts. Van Cleave (personal communication) has a record of infection of *Pleurocera* in Michigan (locality unnamed) by a species of *Neoechinorhynchus* and of an unidentified form from the same genus of snails in central Illinois. In *Pleurocera* the infection was likewise very common, running around 50 per cent in various locations. The snails were collected in the late summer of 1938.—S. C. Whitlock, *Dept. of Conservation, State of Michigan, Lansing.*

PROPHYLAXIS OF MICE AGAINST EXPERIMENTAL *TRYPANOSOMA EQUIPERDUM* INFECTION BY THE PERIODIC ADMINISTRATION OF GERMANIN

Germanin (Bayer 205) exerts a prophylactic effect in both animals and man against infection with certain species of trypanosomes. In man, an injection of one gram or more of the drug has been found to protect against natural infection with *Trypanosoma gambiense* in a heavily endemic area for at least three months (Duke, 1934, Ann. Trop. Med. and Parasitol. **28**: 535), and if injections of one gram be repeated every third month, protection is assured over long periods (Orlovitch, 1937, Ann. Soc. Belge Med. Trop. **17**: 353). In experimental animals, similarly, the drug manifests prophylactic potentialities against infection either by syringe or by the bite of infected flies. The vast majority of these experiments in animals, which have been summarized by Corson (Ann. Trop. Med. and Parasitol. **28**: 535), have dealt with the protection conferred by a single inoculation of the drug. In the work reported in the present paper, we have attempted to find a dose of germanin which, injected into mice periodically at intervals of one month, will completely protect these animals from infection in spite of the repeated inoculation of *T. equiperdum*.

A preliminary test with a few mice had indicated that these animals were generally resistant to *T. equiperdum* for between 21 and 35 days after receiving intraperitoneally 1 mgm of germanin. In the first effort, therefore, a dose of 1 mgm of the drug per 20 gm mouse was employed. This amount was found to be insufficient regularly to protect the animals, since half of the 20 mice given this dose of drug succumbed to infection when inoculated with 10,000 *T. equiperdum* three weeks after the drug was administered.

The experiment was then repeated upon 16 mice, this time, however, 2 mgm of the drug being administered per 20 gm mouse. These animals also were injected with 10,000 *T. equiperdum* 3 weeks after the drug was given. None showed the presence of trypanosomes during the ensuing 7 days and, at the end of this inter-

val, all were reinjected with 2 mgm of the drug. Again, after 3 weeks, the mice were injected with 10,000 trypanosomes and examined during the ensuing week. This time also the mice proved to be completely resistant to infection. This experiment was continued upon the same animals for six months, 2 mgm of the drug being given intraperitoneally per 20 gm mouse at the beginning of each month, 10,000 *T. equiperdum* being inoculated 3 weeks later, and the mice being examined for infection through the final week of each month. No animal showed parasites at any time, although control mice which had received no drug died regularly four or five days after the infecting dose of trypanosomes was given.

It is concluded that mice injected at six monthly intervals with 2 mgm of germanin per 20 gm body weight are completely protected against repeated infection with 10,000 *T. equiperdum,* inoculated 3 weeks after each injection of the drug. The administration of 1 mgm of germanin per 20 gm mouse weight is below an effective minimum for producing this result.—JAMES T. CULBERTSON, *Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.*

THE RELATIVE WEIGHTS OF TERMITES AND THEIR PROTOZOA

The abundance of intestinal Protozoa in termites of the families Mastotermitidae, Kalotermitidae, and Rhinotermitidae is almost unique among host-parasite relationships. Cleveland (1925, Biol. Bull. **48**: 456) stated that "approximately half the body weight of every worker and nymph, except for a few days after molting, is composed of many kinds of large, intestinal flagellates"; and again (1926, Quart. Rev. Biol. 1: 53), "the protozoa in these insects weigh almost as much as the insects themselves." In the public press the statement has several times been published (without Cleveland's qualification) that one-half of the entire weight of a termite may be made up of protozoa. The attention of one of us has been called to several of these statements by Dr. T. E. Snyder, and as it has become a matter of general interest, an attempt was made to determine its validity.

The termites used were *Zootermopsis angusticollis* Hagen and *Z. nevadensis* Hagen. Both animals freshly removed from the wood, and animals which had been kept for varying lengths of time on wood and damp filter paper were used. The technique adopted was as follows. Groups of twelve to twenty-five termites, depending on their size, were weighed. The entire gut and attached tissues were then removed and the remaining portions of the termite bodies were weighed again.

The gut was placed in water or saline solution and teased apart with needles to extract the contents. The solution was pipetted off, together with the protozoa and debris, and the process repeated until essentially all microorganisms were removed. This was occasionally facilitated by letting the material stand in a warm room for a few hours, causing fairly complete cytolysis of the remaining organisms. After the final washings, the remains of the tissue were transferred to a small square of dry absorbent muslin, fastened over the opening of a hand-centrifuge tube, and whirled until any excess moisture had been removed. As much of the tissue as could conveniently be scraped off was transferred to a tared slide and weighed. The sum of these gut weights and the weights of the remains of the eviscerated termites was then compared with the original weights of the live termites, the results being expressed as a percentage of the original weight. Subtracting this value from 100 gives the percentage of the body weight of the termite constituted by the contents of the digestive tract. The results obtained in a series of observations on various stages of the termites used are given in the accompanying table.

It should be pointed out that the technique of the experiments was such that the portion of the body weight attributed to the protozoa of the gut is a maximum, i.e., the true value is probably lower than that given in the table. The few flagellates which might have been left in the tissue after the drastic treatment to which it had been subjected were more than counterbalanced by the termite tissue lost in the manipulations, the non-protozoan matter of the gut contents (wood fragments, bacteria, fluid), and the evaporation loss of the termites from which the gut had been removed, in the interval before weighing. It is to be expected on these

TABLE 1.—*Data on weight (in grams) of termites and their protozoa*

	Group used	Live weight	Weight minus gut	Weight of gut residue	Maximum per cent of protozoa
Z. angusticollis	1 12 seventh instar wingpad nymphs	0.581	0.382	....	34
	2 16 wingpad nymphs	0.925	0.553	0.059	34
	3 24 third-fifth instar nymphs	0.363	0.236	0.016	31
	4 13 mature soldiers	1.497	1.209	0.044	16
	5 12 mature soldiers	1.472	1.137	0.055	19
Z. nevadensis	6 16 wingpad nymphs	0.707	0.415	0.037	36
	7 17 fifth-seventh instar nymphs	0.389	0.203	0.045	36
	8 16 mature soldiers	0.825	0.652	0.034	17

The specimens of *Z. nevadensis* used were extracted from the native log. Those of *Z. angusticollis* had been kept on wood and damp filter paper. The animals of group 3 had been extracted from the wood only a week previously.

grounds that the actual portion of the body weight contributed by the protozoan fauna is several per cent lower than indicated in the table. It is probable therefore that less than one-third of the body weight of a nymph of these species is composed of protozoa.

The figures given here for the proportion of the gut contents are considerably higher than those published by Hungate (1939, Ecology **20**: 244). Presumably, he used nymphs, though the caste is not stated. He estimated that in *Z. angusticollis* the gut contents containing the protozoa, but excluding the wood pellets in the posterior part, amounts to one-seventh to one-fourth of the total weight. Hungate extracted the protozoa by squeezing the abdomen. Dissection of the gut probably gives more complete removal of the contents, so that use of that method would be expected to give a higher percentage.

Many termites in a colony will, of course, have a smaller proportion of protozoa. That is true of soldiers, with a heavier body; of nymphs for a period following the molt, before the normal fauna has been restored; of nymphs with wing pads in a late stage before the final molt; and of imagos.

Aid from the United States Works Progress Administration (Official Project No. 465-03-3-192) is hereby acknowledged.—LEONARD I. KATZIN AND HAROLD KIRBY, JR., *Department of Zoology, University of California.*

A NOTE ON THE PREVALENCE OF INTESTINAL HELMINTHS IN ORANGE COUNTY, NORTH CAROLINA

Fecal specimens were collected from children in the various white and negro rural grade schools in Orange County, North Carolina. The stools were examined by the salt-flotation method. In order to ascertain the approximate number of worms present, Stoll dilution egg-counts (Stoll, N. R., and W. C. Hausheer, 1926, Am. J. Hyg. **6**: 134–145) were made on those specimens containing hookworm or ascaris eggs.

Fecal specimens from 661 school children were examined. Of these, thirty-four or 5.1 per cent contained helminth eggs. The types of helminths found are shown in Table 1. One person only was found with a hookworm infection, an 0.2 per cent infection rate. This single infection occurred in a white girl fifteen years of age. From egg counts made on her feces, it is estimated that she harbored only eight hookworms.

Single infections with *Trichuris trichiura, Hymenolepis nana* and *Hymenolepis diminuta* were present. One infection with *Enterobius vermicularis* was dis-

TABLE 1.—*Intestinal helminths present in school children examined from Orange County, North Carolina*

Parasite	Number of persons infected			
	White	Negro	Unknown	Total
Ascaris lumbricoides	5	22	2	29
Necator americanus	1	..	..	1
Trichuris trichiura	..	1	..	1
Enterobius vermicularis	1	..	..	1
Hymenolepis nana	..	1	..	1
Hymenolepis diminuta	..	1	..	1
Total	7	25	2	34

covered. As this parasite usually does not expel its eggs until it migrates out of the human intestine, fecal examinations are not an accurate index of its prevalence.

By far the most prevalent intestinal helminth found was *Ascaris lumbricoides*. An analysis of the incidence of infection according to age and color groups is given in Table 2. It is seen that there were twenty-nine infections with ascaris

TABLE 2.—*Incidence of infection with* Ascaris lumbricoides *among school children of Orange County, N. C.*

Age	White			Negro			Total		
	No. exam.	No. pos.	%	No. exam.	No. pos.	%	No. exam.	No. pos.	%
5–9	191	3	1.6	105	14	13.3	296	17	5.7
10–14	191	2	1.1	115	7	6.1	306	9	2.9
15–19	36	0	0.0	23	1	4.3	59	1	1.7
Total	418	5	1.2	243	22	9.1	661	27*	4.1

* No information on two cases, making total of 29 or 4.4 per cent.

among the 661 persons examined, an incidence of 4.4 per cent. The rate of infection among the total series of colored persons, 9.1 per cent, was considerably greater than that among the whites, 1.2 per cent. The age group 5–9 years, showed the highest incidence of infection, 5.7 per cent in the combined groups of white and colored as compared with 2.9 per cent in the age group 10–14 years, and with 1.7 per cent in the age group 15–19 years. There was a marked incidence variation by age among the colored children as shown by an infection rate of 13.3 per cent in the age group 5–9 years, which was more than twice that of the age group 10–14 years, and approximately three times that of the age group 15–19 years.

As regards sex distribution of ascaris infection, an analysis of the entire series of white and colored children indicated an approximately equal distribution among males and females.

The intensity of infection with ascaris among the white and colored children was estimated by means of the Stoll dilution egg-count. The whites averaged 32 ascarids per infected person while the negroes' average worm burden was 23.—H. W. BROWN AND A. J. SHELDON, *Division of Public Health, School of Medicine, University of North Carolina, Chapel Hill, North Carolina.*

THE EFFECT OF COLCHICINE ON BIRD MALARIA

The recent work of Brues and Cohen (1936, Biochem. J. **30**: 1363–1368), Allen, Smith, and Gardner (1937, Am. J. Anat. **61**: 321–341), Lahr and Riddle (1938, Am. J. Physiol. **123**: 614–619) and others, have shown that colchicine arrests cell division at metaphase by inhibiting the formation of an achromatic spindle. The experiments reported here were undertaken to test the effects of colchicine upon *Plasmodium relictum* of the pigeon. If cell division were arrested during schizogony, then it would readily follow that the stages of development in the treated pigeons would lag behind those in the untreated birds. Also, the total number of parasites would be smaller in those receiving the drug.

Experiment I. Nine pigeons were divided into 3 groups of 3 birds each. The

birds were given equal amounts of parasitized blood from a common donor. Intravenous inoculations were made simultaneously, and smears were made daily for 10 days following these inoculations.

Group 1 was kept as a control. Group 2 received intravenously 0.6 mgm of colchicine, dissolved in 0.5 cc of distilled water, immediately following the inoculation with the malaria. This dose was given daily for 4 days. The parasites appeared in Group 2, as well as in the other birds, on the third day. The parasites in this group were similar in number and appearance to the parasites in Groups 1 and 3 which had not received the drug.

The treatment of Group 3 was instituted on the third day when the malarial parasites appeared in the blood stream. This group received 0.6 mgm of colchicine intravenously daily for 4 days. These birds had a typical course of malaria and at no time could any quantitative difference be detected between the colchicine-treated pigeons and the controls.

Experiment II. In order to find if the colchicine had any effect on the malaria which had not been detected by making smears every 24 hours, another experiment was carried out in which smears were made every 3 hours for 4 days after the appearance of the parasites. Lahr and Riddle (1938) found that 0.6 mgm of colchicine given intramuscularly became effective within 6 hours and prevented division beyond the metaphase, in the pigeon crop epithelium, for 16 to 18 hours. Similar tests with our sample of the drug gave comparable results. Consequently, in this experiment, the colchicine was given about 8 hours before the trophozoites were due to show nuclear division. Thus, if nuclear division were delayed, it could be readily detected in the counts made at 3 hour intervals.

The 4 birds used received equal inoculations of infected blood as described in Exper. I. On the day that the parasites became patent, 2 of the pigeons received intramuscularly a single dose of 0.6 mgm of colchicine. Two birds were kept as controls.

The 3-hour counts of the parasites over a four-day period revealed a high synchronicity that was identical in both the controls and the pigeons receiving intramuscular colchicine. The time of sporulation remained the same in both groups. The intensity of the infections was similar and no modification of the schizogonic cycle occurred which could be attributed to the colchicine.

Under the conditions of these experiments, there were no retarding effects upon the incubation period or upon the division of *P. relictum* in the pigeon, when colchicine was given either intramuscularly or intravenously.

Colchicine, as has been shown by many observers, specifically inhibits the formation of an achromatic spindle in many types of cells. If such an achromatic figure exists in bird malaria, it is, according to our experiments, not affected by colchicine. On the other hand, the non-effectiveness of colchicine may suggest the absence of such a division figure in avian malaria.—G. Robert Coatney and Martin D. Young, *Field Investigations of Malaria, National Institute of Health, U. S. Public Health Service.*

INFECTIVITY OF *PROSTHOGONIMUS MACRORCHIS* MACY FOR THE COMMON RING-NECKED PHEASANT

On April 15, 1938, 14 dragonfly naiads belonging to the genus *Tetragoneuria* were fed to a female ring-necked pheasant (*Phasianus colchicus torquatus*). The naiads had been collected from Wintergreen Lake on the W. K. Kellogg Bird Sanctuary near Battle Creek, Michigan. Of 30 collected, six were dissected and found to be infected with metacercaria of the fluke *Prosthogonimus macrorchis* Macy, 1934, as determined by previous feedings (Gower, 1938, Mich. St. College, Exper. Sta. Mem. 3, pp. 13–14). In view of the versatility of this species of fluke in infecting various birds (Macy, 1934, Univ. Minnesota Exper. Sta. Tech. Bull. No. 98), the infection of the pheasant in this case was looked upon merely as a routine procedure. However, when the bird was autopsied 12 days later, no trace of the worms was found. The oviduct was at its physiological peak, and a maximum infection would have been the logical expectation.

In order to check the results of this single feeding, naiads were again collected

from Wintergreen Lake on April 28, 1939. Because of the limited number available, only one naiad was dissected. It contained three cysts. The remaining dragonflies were fed to two female pheasants and a bantam hen, the latter being a control. One of the pheasants and the bantam received seven, and the other pheasant six naiads. The three birds were known to be laying at the time of infection, so that more or less ideal conditions were present for infection. At autopsy, eight days after feeding, the control bantam was found to be infected with 15 medium-sized, fully mature *P. macrorchis*. She had laid only one egg after the feeding, and at the time of autopsy the oviduct and ovary showed no signs of functioning. One of the pheasants was infected with a single worm, slightly larger than those from the bantam, and the other pheasant was negative.

These results seem to indicate that the ring-necked pheasant is an extremely refractory host for the oviduct fluke. In view of the ease of infecting chickens with the fluke, it would seem quite logical that they might infect the female of other gallinaceous birds equally well, but apparently the pheasant is an exception. Two previous instances of flukes of this genus occurring in birds of the genus *Phasianus* are recorded: *Prosthogonimus ovatus* has been taken from the oviduct of *Phasianus gallus,* and *P. karausiaki* from the bursa of *Phasianus mongolicus turkestanicus.*

By nature of their habits, pheasants would stand a fair chance of infection in nature, provided they were easily susceptible to infection by this fluke. They are often found near small lakes, and could easily pick up newly emerged dragonfly naiads early in the morning. If such were the case, and the flukes produced a similar physiological derangement of the reproductive organs to that produced in the domestic hen, there might be severe local reductions in the numbers of pheasants due to loss of egg production in areas where *Prosthogonimus* was common. While this experiment was very limited in extent, and much more work should be done before any generalized conclusions can be drawn regarding the host-parasite relationship involved, it does indicate the pheasant to be highly refractory to infection by *P. macrorchis,* at least in the oviduct of the laying female.—W. Carl Gower, *Game Division, Michigan Department of Conservation.*

THE BRANCHIOBDELLIDAE (ANNELIDA) ON CRAYFISHES OF CHAMPAIGN COUNTY, ILLINOIS

During the summer of 1935 the crayfishes of Champaign County, Illinois, were studied to determine the host and habitat restrictions that might exist among the various species of annelids (Branchiobdellidae) living on the crayfishes of the local streams. Crayfishes were collected from all six of the drainage areas of the county and from numerous stations on the small tributary streams. Four species of crayfishes were taken (*Cambarus propinquus, C. virilis, C. blandingi acutus,* and *C. immunis*). None of the burrowing forms (*C. diogenese* and *C. gracilis*) were encountered in the streams during the summer collecting. While each species showed rather definite habitat restrictions, none showed limitations to or exclusions from individual streams except as the distribution was correlated with presence of environmental conditions suitable for the species.

Four species of branchiobdellids were found living on the crayfishes of Champaign County, namely, *Cambarincola macrodonta* Ellis, 1912, *C. vitrea* Ellis, 1919, *C. chirocephala* Ellis, 1919, and *Bdellodrilus illuminatus* (Moore, 1893). For the four species of Branchiobdellidae encountered in this study there is neither host specificity nor fixed limit of stream distribution within the county.—Charles R. Evans, *Clinton, Ill.*

RELATION OF SIZE TO SEX IN PUPAE OF *AEDES AEGYPTI* (LINN.), *A. TRISERIATUS* (SAY) AND *A. VEXANS* MEIGEN

While rearing the mosquito, *Aedes aegypti* (Linn.), the writer noticed that the pupae seemed to be of two distinct sizes. As the female adult of this species is considerably larger than the male, this suggested that it might be possible to separate the sexes in the pupal stage according to size. It proved to be an easy matter to do so, the larger pupae all producing females and the smaller, males.

It seemed unlikely that so obvious a fact had escaped the attention of the many investigators who have studied this important species, but a review of the literature has yielded no reference to it nor any indication that it is generally appreciated.

Shannon and Putnam (1934, Proc. Entom. Soc. Washington, **36**: 185–216) describe their procedure for handling this mosquito thus: "Each morning the pupae are removed (from rearing jars) and isolated in individual Wassermann tubes. The tubes are examined daily for adults. The males are discarded and the females placed in experimental cages."

Brett (1938, Tr. Roy. Soc. Trop. Med. and Hyg. **32**: 113–124) states of this species, "Fifty females were placed in each cage, and there were usually two or three males as well, as it was found extremely difficult to exclude all of these."

From these references it is evident that an easy method of separating the sexes as the pupae are taken from the rearing jars would be of advantage to the experimenter who is interested in the females only.

To check the difference in size more accurately a naturally infested jar of larvae was set aside and the pupae appearing each day for six days were measured. Measurements were made of the greatest length of the cephalothorax of each pupa. Then it was isolated so the sex could be checked upon emergence. There were 43 males and 22 females. The results are recorded in the table as "Set No. 1."

TABLE 1.—*Measurements of the cephalothorax of mosquito pupae*

Set No.	Males		Females		Difference	Per cent Difference
	Mean length of cephalothorax	Range	Mean length of cephalothorax	Range		
1	41.1* ± 0.22†	38–43	49.9 ± 0.26	48–53	8.8 ± 0.34	19
2	40.6 ± 0.62	38–42	48.3 ± 0.43	45–51	7.7 ± 0.77	17
3	34.9 ± 0.64	29–38	38.7 ± 0.58	36–42	3.8 ± 0.86	10
4	37.6 ± 0.44	36–40	41.6 ± 0.75	36–46	4.0 ± 0.87	10
5	48.3 ± 0.74	43–59‡	57.5 ± 0.81	51–60	9.1 ± 1.10	17
6	47.8 ± 0.22	44–53	49.0 ± 0.48	45–53	1.2 ± 0.53	2

* Unit = 50 μ. † Standard error. ‡ Only one over 51.

Another group of *Aedes aegypti* pupae from eggs laid in captivity was measured. There were 29 males and 37 females. The results appear as "Set No. 2."

As it is known that the size of mosquito pupae is extremely variable depending on crowding of the larvae it seemed important to test the effect of crowding on the sexual difference. About 50 one-day-old larvae were placed in a small vial and fed sparingly. After a prolonged larval period they pupated. These pupae were measured in two groups: (1) those appearing by March 10; and (2) those appearing March 11 and 12. In the former group there were 8 females and 14 males and in the latter 13 females and 16 males. The results appear as "Set No. 3" and "Set No. 4," respectively.

Three points seem worth noting here: (1) The female size was reduced below the normal male size; (2) the females were reduced proportionately more than the males; (3) although a difference in the sexes remains the ranges are no longer discontinuous. For these reasons the proposed technique for separation can be used only on pupae that have developed under favorable conditions and under the same conditions of crowding.

Measurements were made on two other species, *Aedes triseriatus* (Say) and *Aedes vexans* Meigen. In each instance measurements were made on groups of pupae appearing in the same jar within a period of 24 hours. All the larvae of each group had been collected from one source. The results of measurements on 36 males and 28 females of *Aedes triseriatus* are given as "Set No. 5." The results of measurements of 54 males and 52 females of *Aedes vexans* are given as "Set No. 6."

Aedes triseriatus shows a difference almost as great as that of *Aedes aegypti* but the size ranges of the sexes overlap somewhat. *Aedes vexans*, on the other hand, shows little difference with almost identical size ranges.—WILLIAM CANTRELL, *Zoology Department, University of Georgia, Athens, Ga.*

Edwin Linton (1855–1939)

IN MEMORIAM

Edwin Linton (1855–1939)

In the death of Edwin Linton on June 5 American zoology lost a grand old man. When the accident occurred that led to his death six months later he was well advanced in his 84th year. The circumstances illustrate so well the degree to which he retained his mental and physical vigor and his courage, self-control and good judgment that they deserve recording. Last Thanksgiving Day he was attacked by a rabid dog. Instead of running he caught the dog by the neck and succeeded in avoiding a bite, but in the struggle was thrown and suffered a fractured femur. Even then he continued to hold the dog until it was removed by a veterinarian. He further showed his mastery of the situation by refusing all offers to be moved but insisted upon lying as he was until the arrival of an ambulance, insuring expert handling. Although in excellent health at the time, the long confinement in the hospital, with the shock and the reaction to the Pasteur treatment given as a precautionary measure, so weakened him that he succumbed to an attack of pneumonia.

The general facts of Doctor Linton's life and his chronology may be found in "American Men of Science" and "Who's Who." Here only a brief chronicle of some of the characteristics of the man and his work will be attempted. His serious work in zoology began in 1881 when he went to Yale as a student of Professor A. E. Verrill. Professor Spencer F. Baird was then searching the coast for a suitable location for a fisheries research laboratory. Verrill was the invertebrate zoologist of the investigating parties and invited Linton to join him as assistant during the summer of 1882 and subsequently when they were working at Woods Hole. In the summer of 1884 Professor Baird encouraged Linton to take as his specialty the entozoa of marine fishes, a then virgin field. Except when he was engaged in similar work elsewhere (Yellowstone Park, 1899; Beaufort, 1901, 1902; Bermudas, 1903; Tortugas, 1906–08) and until 1935 Doctor Linton was a regular summer investigator at Woods Hole where a large room was reserved for him in the Fisheries Laboratory. His duties as Professor of Geology and Biology at Washington and Jefferson College from 1882 to 1920 left him little time for research during the academic year, but he devoted his summers enthusiastically to his parasitological researches.

A real pioneer in his field of research Doctor Linton antedated such helminthological elders as Doctors Ward and Stiles. Doctor Joseph Leidy was practically the only active American worker in animal para-

sitology and Leidy was little concerned with the parasites of marine fishes. How rapid progress has been in fifty years is shown by the fact that we now have a thriving American Society of Parasitologists with a membership of over 400, a large part of whom are contributing investigators, and that the federal government is engaged in extensive measures of inspection and control of the helminths of man and the domestic animals. At the time of his death Doctor Linton is believed to have been the oldest active worker in this group.

Doctor Linton's scientific publication began in 1887 in two brief papers, one in the "Proceedings of the U. S. National Museum" on a trematode identified as *Distomum ovatum* Rudolphi, found in the albumen of a hen's egg, and the other in the "American Naturalist" on two forms of cestode embryos. These showed those qualities of accurate observation, careful description, scholarly thoroughness and readable style which have characterized his research and publication during the subsequent half-century. The interest in parasitic worms thus early established has held with him ever since and his latest paper, one of several unpublished manuscripts, is entitled, "Trematodes of Fishes Mainly of the Woods Hole Region."

During the period between these terminal publications a continuous if not copious stream of papers numbering somewhat over 100 flowed from Doctor Linton's pen. Nearly all of these are helminthological and cover the whole field of verminous parasites of fishes and birds. About 30 deal with the adult cestodes of sharks, a smaller number with those of birds and both adult and larval cestodes of smaller fishes and a still smaller number with trematodes. The remainder are made up of an occasional paper on nematodes, Acanthocephali and even on protozoan parasites of fishes, the food of fishes and the habits of fishes. His papers are well illustrated, often by the artistic hand of Mrs. Linton.

Doctor Linton's technical papers are essentially systematic and abound in descriptions of new species and in host records and descriptive notes on known ones. With regard to genera he was properly conservative. In a hasty examination of his papers only eight proposed genera were noted, although some may have been overlooked. Nor am I aware that he established any larger taxonomic groups. His real interest, as he confessed to the writer, was in life histories. It was this that led him to study especially the cestodes of bony fishes, sharks and fish-eating birds in relation to one another. While he carried on no feeding experiments, which would generally be very difficult or impossible with marine fishes, he did succeed by morphological studies in correlating some cysticercoid forms of smaller fishes with the adults in the sharks and birds which prey upon them. For example in 1890 he studied the tapeworm known as *Dibothrium cordiceps* Leidy, the larval stages of which infect the trout of Yellowstone Lake and showed that the infection comes from the white

pelicans that breed on Molly Island which are parasitized by the mature worms. He was also much concerned with the practical bearings of parasitology and at times gave his services to the solution of problems of this kind affecting the fisheries and human welfare.

Whatever final valuation specialists may place upon his systematic work it seems certain that he has provided a valuable store of carefully observed facts relating to anatomy, host-parasite relations, ecology and life histories that cannot but be useful to future taxonomists. That his work has received recognition is attested by the award to him of two medals, election to a foreign correspondentship, and other honors.

Of recent years Doctor Linton's thoughts turned more toward evolutionary philosophy and polemics, largely stimulated by the ignorance and bigotry brought to light by the Scopes trial and the wave of discussion and popular interest resulting therefrom. This was crystallized in his address as Vice-President of Section F of the American Association for the Advancement of Science in 1925 and in a course of lectures delivered by invitation at the Summer School of the University of Virginia at Mountain Lake in 1936. His less technical articles in foreign and home periodicals and in memorial volumes were written mostly upon invitation. His retentive memory, excellent delivery, clear diction, inimitable sense of humor and dramatic sense made him in demand for the delivery of his address, particularly upon anniversary occasions when reminiscences were particularly appropriate. Among the best of these were his address at the unveiling of the Baird memorial tablet at Woods Hole and his Reminiscences of a Helminthologist.

After his retirement as Emeritus Professor in 1920, Doctor and Mrs. Linton accompanied their daughter Eleanor and her husband, Doctor Eliot R. Clark, successively to the Universities of Missouri, Georgia and Pennsylvania. To each of these they were welcomed warmly and given honorary status. At Pennsylvania since 1926 Doctor Linton has occupied a research room in the Zoological Laboratory as an honorary fellow and until his hospitalization he seldom missed a day's work. For us in the Zoological Department it was a happy circumstance that brought Doctor Linton to Pennsylvania. He has been a stimulus and a shining example to students and faculty members alike. His steadfastness, lively interest in persons and affairs, his cheerfulness, humor and general good fellowship endeared him to all, and his death has left a deep chasm bridged by many treasured memories.—J. PERCY MOORE, *University of Pennsylvania.*

AMERICAN SOCIETY OF PARASITOLOGISTS

PRELIMINARY ANNOUNCEMENT OF THE FIFTEENTH ANNUAL MEETING

THURSDAY, FRIDAY AND SATURDAY, DECEMBER 28–30, 1939
COLUMBUS, OHIO

Pursuant to the action taken at the fourteenth annual meeting in Richmond, Va., the American Society of Parasitologists will convene for a three-day program in conjunction with the meeting of the American Association for the Advancement of Science in Columbus, Ohio. The Deshler-Wallich Hotel will be headquarters for the Society, and the program sessions will be held in Townshend Hall on the campus of Ohio State University.

The first and third days will be devoted to the reading of contributed papers. At the conclusion of the morning session of the second day, the presidential address will be delivered by Dr. Horace W. Stunkard on the subject, "Life History Studies and the Development of Parasitology." The annual Parasitologists' Luncheon and business meeting of the Society will follow the presidential address, and the demonstration program and tea will be held in the afternoon. Each year the demonstration program has become a more important feature of the Parasitology meetings, and members are urged to present papers by this method if possible.

The call for papers has already been sent to members of the Society. In order that the abstracts be published and mailed to the membership before the meetings, it is necessary that they be received in the office of the Secretary not later than Friday, November 3, 1939. Members are hereby reminded of this date, and are requested to be prompt in the submission of papers for the program.

O. R. McCOY, *Secretary*.